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生物進化研究のモデル生物群としてのショウジョウバエ

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はじめに

次世代シークエンサーの実用化が現実のものとなりつつある現在，ゲノムベースでの比較研究が，生物進化研究の新しい扉を開きつつある．ショウジョウバエ類は，モデル生物であるキイロショウジョウバエ *Drosophila melanogaster* を中心に，現代生物進化学の重要な概念の確立をもたらした数々の研究に用いられてきた．また，Drosophila 12 Genomes Project は，キイロショウジョウバエ以外の 11 種を含む全 12 種のゲノム配列を解読・公開して，比較ゲノム時代の扉をいち早く開けた．このような時代背景にあって，これからの生物進化研究は，特定のモデル生物種に限らない，多くの野生種を対象にしたゲノムベースの実証的研究が可能となり，新たな学問的地平を拓く挑戦が始まるにちがいない．系統分類学の分野では，主な分類群のゲノム配列を骨格とした DNA 配列の巨大データに基づく網羅的な分子系統解析が可能となり，その結果に基づいて，従来の分類体系の見直しが進むだろう．また，種々の形態の発生・遺伝機構が明らかにされ，生物が示す形態変異の相同性と多様性に関する進化的理解が進むだろう．行動・生理・生態学分野では，これまで難しかった野生種の適応的形質の分子・遺伝機構を解明する道が拓け，生物進化学は，進化の結果としての形質を比較してそのプロセスを類推する学問から，その素過程をも解明する学問に脱皮するだろう．そして，メタゲノム解析技術の導入は，群集・生態系分子生態学の道を拓く強力な武器となるにちがいない．

日本のショウジョウバエ研究は，モデル生物としてのキイロショウジョウバエに限らず，多くの野生種を研究対象としてきた長い伝統を有しており，今や世界でも有数の野生ショウジョウバエ研究の広がりと深みを持っている．例えば，アジアのショウジョウバエ類に関する系統分類・生物地理学，野生ショウジョウバエ類の季節・気候適応の生理・生態学，野生ショウジョウバエと寄主・寄生者との相互作用，野生ショウジョウバエ群集の構造・種多様性の緯度変異などの研究成果は，ゲノムベースの新たな生物進化学に多くの重要な研究課題を提供する可能性がある情報の宝庫である．

本特集は，2010 年 9 月 22-23 日に北海道大学で開催された研究集会「ショウジョウバエ研究のいまとこれから ── 特に，キイロショウジョウバエ以外の研究に注目して ──」（セッション 1 ：ショウジョウバエ進化研究のこれから〜ポスト 12 ゲノムの新展開〜，第 82 回日本遺伝学会ワークショップ；セッション 2 ：生物進化研究のモデル生物群としてのショウジョウバエ，低温科学研究所共同研究集会）で発表された論文を中心に，さらに関連分野の論文を募り，17 件の論文を掲載している．これらの論文が，野生ショウジョウバエおよびキイロショウジョウバエを材料とした分類，形態，行動，生態，生理，遺伝，分子生物学の「いま」を総括し，「これから」の比較ゲノム時代に向けた新たな発想，研究課題，展望を拓く一里塚となることを期待したい．

本特集の編集は戸田正憲が担当した．最後にこの場を借りて，論文を投稿された全ての著者と，論文の審査を引き受けていただいた査読者の皆様に感謝いたします．

「低温科学」第 69 巻編集責任者　戸田正憲

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ショウジョウバエ分子系統学研究の最前線

加藤　　徹[1)]

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ショウジョウバエ科の系統関係については古くから注目され，これまでにいくつかの古典的な研究が知られている．中でも，Throckmorton は 1975 年に内部形態や地理的分布などに基づいたショウジョウバエ科の系統に関する仮説を提唱し，多くの研究者に広く受け入れられてきた．しかし，その後，Grimaldi（1990）は数多くの外部形態を用いて分岐学的解析を行い，Throckmorton の説と大きく異なる仮説を提唱した．それ以来，ショウジョウバエ科の系統関係を分子系統学的見地から再検討しようとする試みが，これまで数多くなされている．興味深いことに，分子系統解析に基づく最近の研究結果は，後から提唱された Grimaldi の仮説よりも，むしろ古典的な Throckmorton の仮説に近い傾向にある．しかしながら，ハワイ産ショウジョウバエを含むいくつかの重要な分類群の系統関係については，分子系統解析から，両者何れの仮説とも異なる独自の見解が新たに得られている．本稿では，ショウジョウバエにおける最近の分子系統学研究の結果を概説するとともに，ショウジョウバエ系統学の今後の展望について議論する．

Progress and prospects of the molecular phylogenetic studies of Drosophilidae

Toru Katoh[1]

The phylogeny of Drosophilidae has been of great interest, and several classical studies have been made. Among them, one of the most famous studies was Throckmorton (1975), in which he proposed a phylogenetic framework for Drosophilidae based on the comparisons of internal morphology and biogeography. On the other hand, based on a cladistic analysis of external morphology, Grimaldi (1990) put forward another hypothesis that was substantially different from that of Throckmorton (1975). Since then, many molecular approaches have been undertaken in order to solve this problem. It is noteworthy that many phylogenetic studies using molecular data on the whole are in agreement with Throckmorton's classical hypothesis rather than Grimaldi's. Furthermore, these studies also give some additional findings that are different from either Throckmorton's or Grimaldi's. I review these results and then discus prospects of phylogenetic studies on Drosophilidae.

1．はじめに

ショウジョウバエは，ショウジョウバエ科（Family Drosophilidae）に属する双翅目昆虫の総称を指す（図 1）．これらは，目が赤い，お酒に集まる習性があることから，顔が赤くて酒好きの妖怪「猩々」にちなんで名付けられたとされる（森脇，1979）．現在までに 4,000 種以上が記載され，極地を除く世界各地に広く分布する（Bächli, 1999–2010; Toda, 2006–2010）．また，その生活も，果物食，樹液食，草本食，キノコ食と非常に多岐に渡る．

一般に，ショウジョウバエと言えば，キイロショウジョウバエ（*Drosophila melanogaster*）を指すことが多く，実際，本種はさまざまな研究分野のモデル生物として確固たる地位を築いている．しかし，キイロショウジョウバエ以外にも，これまでさまざまなショウジョウバエ種が，遺伝学，行動学，生態学などの研究分野を含む生物学研究に広く用いられてきている（Powell, 1997）．そして，2007 年には 12 種のショウジョウバエ種について全ゲノムが決定され（Drosophila 12 Genomes Consortium, 2007），比較研究の対象として重要性が増している．このように，ショウジョウバエが自然界で保有している遺伝的変異，あるいは種間のさまざまな生物学的特徴における差異は，生命現象の探求のための得難い資産となる．そして，それらの多様性に着目して研究を拡大する際，ショウジョウバエ科の系統進化

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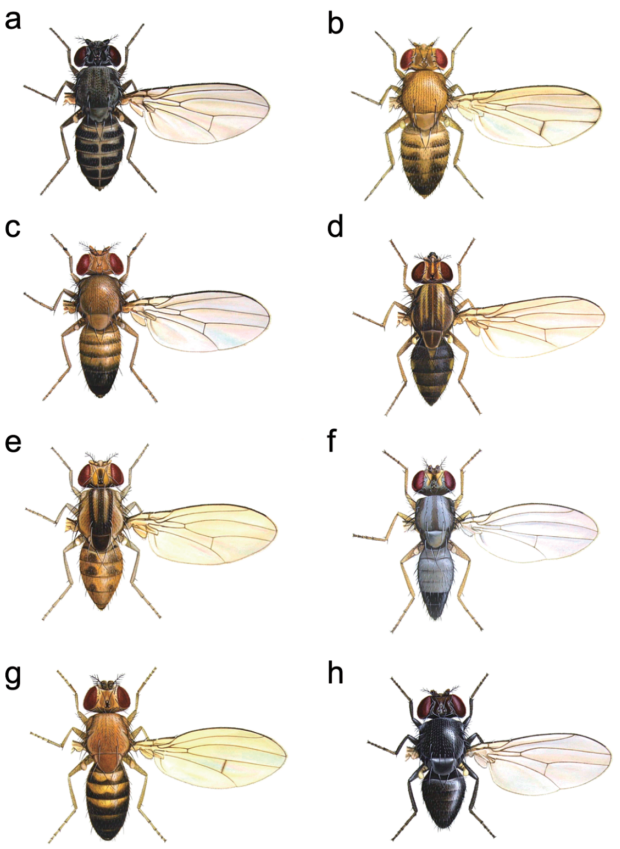

図1：ショウジョウバエ科の中のいくつかの種．a：*Drosophila* (*Drosophila*) *hydei*. b：*D.* (*Drosophila*) *immigrans*. c：*D.* (*Sophophora*) *simulans*. d：*Zaprionus grandis*. e：*Hirtodrosophila trilineata*. f：*Scaptomyza pallida*. g：*Lordiphosa collinella*. h：*Scaptodrosophila coracina*.

に関する情報は，それ自身が興味深い知見であるのみならず，ショウジョウバエを用いたあらゆる研究の重要な基盤となる．

そして，近年の分子系統解析学的手法の発達に伴い，ショウジョウバエの系統関係に関する我々の理解は飛躍的に向上した．しかしながら，いくつかの重要な分類群の系統関係については，未だ不明な点が残されている．

本稿では，ショウジョウバエにおけるこれまでの系統学研究の結果を概説するとともに，ショウジョウバエ系統学の今後の展望について議論する．

2．ショウジョウバエの系統に関する二つの仮説

ショウジョウバエ科の系統関係については古くから注目され，これまでにいくつかの古典的な研究が知られている（Sturtevant, 1921, 1942; Patterson and Stone, 1952; Okada, 1956, 1989; Throckmorton, 1962, 1965, 1966, 1975; Wheeler, 1981, 1986）．その中で，最も注目すべき研究の一つとして，Throckmorton（1962, 1965, 1966, 1975）による内部形態を主に用いた一連の研究が挙げられる．

彼は，数多くのショウジョバエについて，雌雄の内部生殖器，および卵の形態的特徴を詳細に観察し，比較を

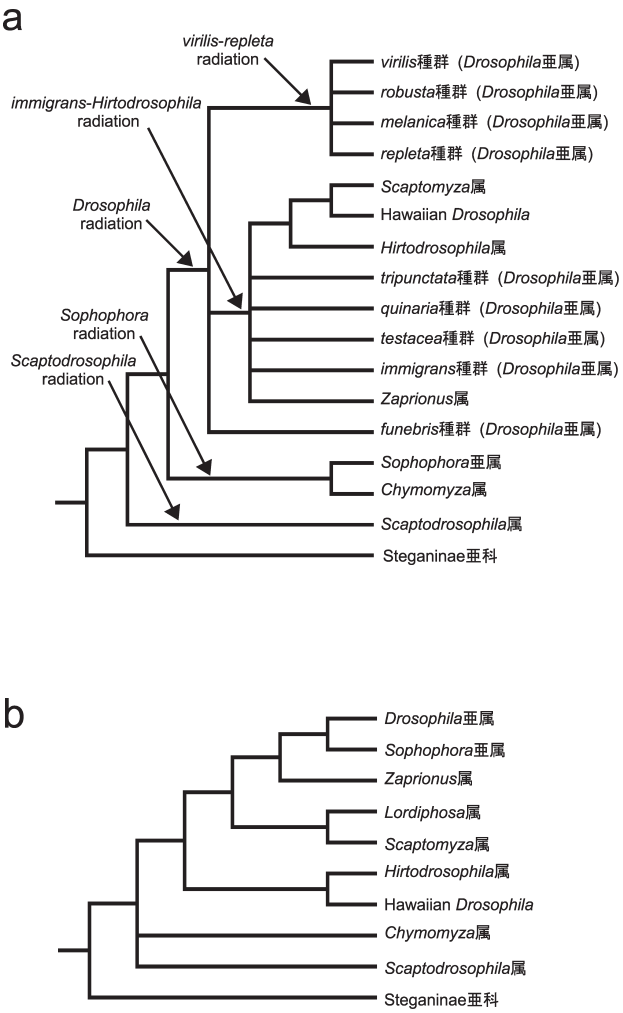

図2：Throckmorton（1975）およびGrimaldi（1990）の仮説に基づく，ショウジョウバエ科の主な分類群間の系統関係．a：Throckmorton（1975）の仮説．b：Grimaldi（1990）の仮説．

行なった（Throckmorton, 1962, 1965, 1966）．そして，得られた知見に生態および生物地理学的情報も加え，ショウジョウバエ科全体の系統進化に関する仮説を1975年に提唱した（図2 a）．

これによると，現在認められるショウジョウバエの系統群は，〝radiation（放散）〟と呼ばれる，特定の時期に分化が急速に生じるイベントが複数回起こったことにより，形成されたと考えられている．その中で，最初に起こったのはsteganine radiationで，この放散では，Steganinae亜科に属するショウジョウバエが分化したとされる．残りの放散は全てdrosophiline radiationに含まれ，その中で*Scaptodrosophila* radaiation，*Sophophora* radiation，および*Drosophila* radiationと呼ばれる放散が順次起こった．そして，*Drosophila* radiationからは*virilis-repleta* radiationおよび*immigrans-Hirtodrosophila* radiationの二つが起こったとされる．

そして，この仮説では，ショウジョウバエ科の中のいくつかの分類群が，必ずしも単系統群を形成しない点で注目される．中でも，*Drosophila*属の中の*Drosophila*

亜属は典型的な側系統群であるとみなされ，いくつかの種群が，同亜属の他の種群よりも別属に近い関係にあることを提唱している．

Throckmortonの仮説は，ショウジョウバエ科全体に渡る包括的なもので，発表当時から多くの研究者に広く受け入れられてきた．しかしながら，その一方で，彼の研究から形質評価の判断基準を理解することは容易でなく，そのため客観的根拠に乏しいという批判も同時に存在した．

Throckmortonの仮説におけるこのような問題をふまえ，Grimaldi（1990）は，ショウジョウバエ成虫における217の外部形態の形質を用いて，最節約法による系統解析（分岐学的解析）を独自に行なった．その結果，得られた系統樹の樹形は，Throckmortonの仮説とは大きく異なるものであった（図2b）．例えば，Throckmortonの仮説では，*Drosophila*亜属は単系統的にまとまらないが，Grimaldiの系統樹では，*Drosophila*亜属は単系統で，*Sophophora*亜属と最も近い姉妹群を形成する．また，Throckmortonの仮説では，Hawaiian *Drosophila*，および*Scaptomyza*属のショウジョウバエは，何れも*immigrans-Hirtodrosophila* radiationにより生じたとされるが，Grimaldiの系統樹では，これらは単系統ではなく独立に生じ，それぞれ別々の系統と姉妹群を形成する．そして，この系統樹を基に，Grimaldiは，*Lordiphosa*，*Hirtodrosophila*，およびHawaiiann *Drosophila*などの分類階級を属に昇格させるなど，いくつかの分類学的再編も同時に行なった．こうして，1990年以降，ショウジョウバエの系統関係については，Throckmorton，およびGrimaldiの仮説という，大きく異なる二つの説が存在するようになった．

3．分子系統解析に基づくショウジョウバエの系統関係

分子系統学は，DNAやタンパクに刻まれた遺伝情報の違いを比較することで，生物の系統を推定しようとする研究分野である．DNA塩基配列の決定が容易でなかった1960–70年代当時は，タンパク質を電気泳動して遺伝的差異を間接的に調べる研究がいくつかの生物群を対象に行われていたが，Throckmortonはこれをショウジョウバエの系統学研究に適用することに一早く注目し，その有用性について論じた総説を報告している（Throckmorton, 1968, 1977）．また，1980年代以降は，幼虫血リンパの免疫凝集反応あるいはDNAハイブリダイゼーションを利用したショウジョウバエの系統学研究がいくつか報告されている（Beverley and Wilson, 1982, 1984; Caccone et al., 1992）．そして，1990年代以降は，分子生物学的手法の発達とコンピュータの処理能力の段階的向上により，DNA塩基配列を用いたショウジョウバエの系統学研究が活発に行なわれている．これらの研究は，いくつかの細かい部分で違いが認められるものの，おおまかには一致する結果を示している（Markow and O'Grady, 2006; van der Linde and Houle, 2008; van der Linde et al., 2010）．

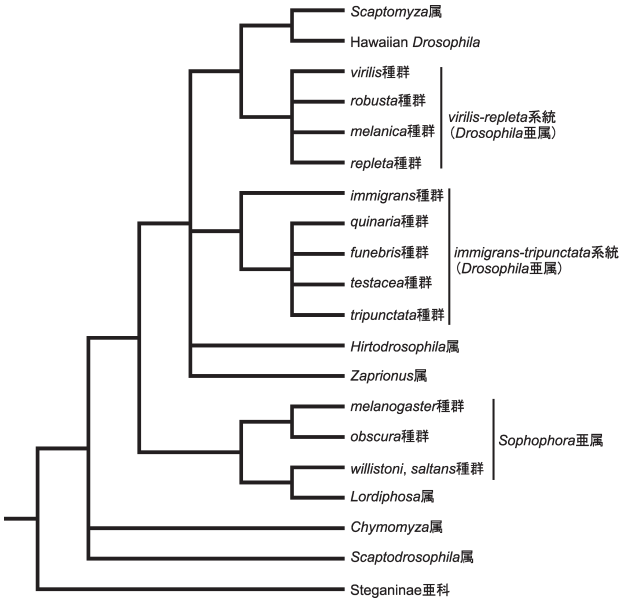

図3：これまでの分子系統学的研究から推定された，ショウジョウバエ科の主な分類群間の系統関係．

そこで，これまでの分子系統解析の結果に基づく，ショウジョウバエ科の主要な分類群間の系統関係をまとめると，図3のようになる．この系統樹を見ると，*Drosophila*属のうち，*Sophophora*亜属と*Drosophila*亜属は姉妹群を形成せず，*Sophophora*亜属の系統がショウジョウバエ亜科の他の属，亜属よりも先に分岐することが特徴的である．また，*Drosophila*亜属についてはそれ自身が単系統的にまとまらず，何れも，*Zaprionus*属，*Hirtodrosophila*属，*Mycodrosophila*属，*Scaptomyza*属，およびHawaiian *Drosophila*などの分類群の側系統群として位置づけられる．従って，分子系統解析に基づくこれまでの研究結果は，後で提唱されたGrimaldiの仮説よりも，むしろThrockmortonの古典的仮説に近い傾向にある．ただし，いくつかの分類群の系統関係については，ThrockmortonあるいはGrimaldiの仮説の何れとも異なる独自の見解が新たに得られている．以下に，これらの内容について，主要な分類群ごとに項目を分けて説明する．

3.1 *Scaptodrosophila*属

*Scaptodrosophila*属はこれまで200種以上が記載され，世界中に分布が認められるが，とりわけオーストラリア大陸での分化が著しい（Bock and Parsons, 1975）．本属は以前，*Drosophila*属の中の一亜属として位置付けられていたことがある（Sturtevant, 1942; Wheeler, 1981）．しかし，Throkmorton（1975）は，これらは

Scaptodrsophila radiationにより，ショウジョウバエ亜科に属する他の多くの系統群に先立って分化した古い系統であるとみなした．また，これと同様の見解は，Grimaldi（1990）の研究からも得られており，その結果をもとに，彼は*Scaptodrosophila*の分類階級を亜属から属に昇格させた．

そして，分子系統解析によるこれまでの研究は，一致して，*Scaptodrosphila*属が系統樹の根元で分岐する樹形を示すことから，両者の見解を支持する結果が得られている（Kwiatowski et al., 1994, 1997; Kwiatowski and Ayala, 1999; Tatarenkov et al., 1999; Katoh et al., 2000; Tarrio et al., 2001; Da Lage et al., 2007; O'Grady and DeSalle, 2008; van der Linde and Houle, 2008; van der Linde et al., 2010）．なお，最近では，これらの研究結果をふまえて，*Drosophila*属およびその周辺の分類群の系統関係を調べる際に，本属がそれらの外群（アウトグループ）として用いられることもある（Robe et al., 2005, 2010; Katoh et al., 2007）．

ただし，本属の内部の系統関係についてはこれまであまり詳しく調べられておらず，その単系統性については疑問がある．実際，O'Grady and DeSalle（2008），およびvan der Linde et al.（2010）による最近の研究において，本属が単系統にならないことが示唆されている．

3.2 *Chymomyza* 属

*Chymomyza*属はこれまで約60種が記載され，新旧大陸の熱帯域を中心に分布が認められる．Throckmorton（1975）によると，本属は*Scaptodrosophila* radiationの後に起こった*Sophophora* radiationで生じたとされる．しかし，多くの分子系統樹において，本属は*Sophophora*亜属との近縁性を示すことはなく，*Sophophora*亜属が分岐する前に系統樹の根元で分岐することが確認されている（Kwiatowski et al., 1994, 1997; Kwiatowski and Ayala, 1999; Tatarenkov et al., 1999; Katoh et al., 2000; Tarrio et al., 2001; O'Grady and DeSalle, 2008; van der Linde and Houle, 2008; van der Linde et al., 2010）．この結果は，*Scaptodrosophila*属と同様，本属が他の主要なショウジョウバエの外群として位置付けられるというGrimaldi（1990）の見解を支持する．

3.3 *Lordiphosa* 属

*Lordiphosa*属はこれまで約60種が記載され，旧北区および東洋区に分布が認められる．本属の系統学的位置についてはこれまで不明な点が多く，*Sophophora*亜属に近縁であるという説（Okada, 1963; Laštovka and Máca, 1978），および*Scaptomyza*属に近縁であるという説（Grimaldi, 1990）が提唱されている．また，本属に分類されている種の中には，かつては*Sophophora*亜属，*Hirtodrosophila*属，および*Drosophila*亜属など，それぞれ異なる分類群に属していたものも含まれている（Laštovka and Máca, 1978; Okada, 1984, 1990; Toda, 1983）．

近年の分子および形態に基づく系統学研究によると，*Lordiphosa*属の主な種群は*Sophophora*亜属に近縁である一方，*tenuicauda*種群については*Lordiphosa*属の他の種群と異なり，*Hirtodrosophila*属や*Mycodrosophila*属に近縁であることが示唆されている（Katoh et al., 2000; Hu and Toda, 2001）．この結果をふまえ，Hu and Toda（2002）は，*tenuicauda*種群を*Lordiphosa*属から*Dichaetophora*属に含めるという分類学的再編を行なった．また，Katoh et al.（2000）は，*Lordiphosa*属の主な種群が，*Sophophora*亜属の中の*willistoni-saltans*系統群と姉妹群を形成することを示し，このことは，*Sophophora*亜属を側系統とみなす点で注目される．

3.4 *Drosophila* 属

*Drosophila*属のショウジョウバエはこれまで1,000種以上が記載され，8亜属（*Chusqueophila*，*Dorsilopha*，*Drosophila*，*Dudaica*，*Phloridosa*，*Psilodorha*，*Siphlodora*，*Sophophora*）に分類されている．そして，そのうちの多くの種は，*Drosophila*亜属あるいは*Sophophora*亜属のどちらかに含まれている．

3.4.1 *Sophophora* 亜属

*Sophophora*亜属はこれまで300種以上が記載され，*melanogaster*種群，*obscura*種群，*willistoni*種群，および*saltans*種群の4つの種群で主に構成される．これらのうち，*melanogaster*種群と*obscura*種群は主に旧大陸で分化した系統，*willistoni*種群と*saltans*種群は新大陸の系統と，大きく二つの系統に分けられる（Pélandakis et al., 1991; Pélandakis and Solignac, 1993; Kwiatowski et al., 1994; Powell and DeSalle, 1995; Kwiatowski and Ayala, 1999; O'Grady and Kidwell, 2002; Remsen and O'Grady, 2002; Da Lage et al., 2007）．

これまでの形態および分子データを用いた多くの研究は，*Sophophora*亜属が単系統群を形成し，*Drosophila*属の中で最初に分化したことを支持する（Throckmorton, 1975; Russo et al., 1995; Tamura et al., 1995; Kwiatowski and Ayala, 1999; Tatarenkov et al., 1999; Tarrio et al., 2001; Remsen and O'Grady, 2002; Robe et al., 2005; Da Lage et al., 2007; Katoh et al., 2007; O'Grady and DeSalle, 2008; van der Linde and Houle, 2008; van der Linde et al., 2010）．ただし，*Lordiphosa*属の系統学的位置をふまえると，これらが必ずしも単系統であるとは言えなくなる（Katoh et al., 2000; Hu and Toda, 2001）．また，核遺伝子を用いたいくつかの分子系統学研究では，本亜属が単系統的にまとまらず，*willistoni-saltans*系統群が，*melanogaster-obscura*系統

群および *Drosophila* 亜属のショウジョウバエの分岐に先立って分岐する樹形を示すことがある（Pélandakis et al., 1991; Pélandakis and Solignac, 1993; Kwiatowski et al., 1994, 1997; Katoh et al., 2000; Tarrio et al., 2001）．これについては，DNA コード領域内のコドン第 3 ポシションにおける塩基組成が，*willistoni-saltans* 系統群と *melanogaster-obscura* 系統群との間で著しく異なることに起因すると考えられている（Anderson et al., 1993; Rodriguez- Trelles et al., 1999; Tarrio et al., 2000, 2001; Powell et al., 2003; Vicario et al., 2007）．

3.4.2 *Drosophila* 亜属

Drosophila 亜属はこれまで 700 種以上が記載されている大きな分類群で，40 以上の種群に分けられている．これまでの研究によると，本亜属は単系統群を形成せず，*Hirtodrosophila* 属，*Mycodrosophila* 属，*Zaprionus* 属，*Samoaia* 属，*Scaptomyza* 属，および Hawaiian *Drosophila* などの分類群を含むいくつかの系統の側系統群として位置付けられる（Throckmorton, 1975; Biverley and Wilson, 1984; DeSalle, 1992; Pélandakis and Solignac, 1993; Thomas and Hunt, 1993; Kwiatowski et al., 1994, 1997; Russo et al., 1995; Tamura et al., 1995; Remsen and DeSalle, 1998; Tatarenkov et al., 1999, 2001; Katoh et al., 2000, 2007; Tarrio et al., 2001; Remsen and O'Grady, 2002; Da Lage et al., 2007; van der Linde and Houle, 2008; van der Linde et al., 2010）．そして，本亜属の中には *virilis-repleta* 系統，および *immigrans-tripunctata* 系統と呼ばれる二つの大きな系統が認められ（Remsen and O'Grady, 2002; Yotoko et al., 2003; Robe et al., 2005; van der Linde and Houle, 2008; van der Linde et al., 2010），これらは *Sophophora* 亜属が最初に分化した後，上記の他の分類群とともにそれぞれ分化したと推定される．

3.4.2.1 *virilis-repleta* 系統

Throckmorton（1975）によると，*virilis-repleta* 系統は，*virilis-repleta* radiation と呼ばれる放散によって生じたと推定されている．この放散は 2 回の放散からなり，最初の放散（primary radiation）は旧大陸を中心に生じ，その後，新大陸で *repleta* radiation と呼ばれる 2 回目の放散が生じたとされる．そして，最初の放散で生じたものには，*virilis* 種群，*robusta* 種群，*melanica* 種群，*carsoni* 種群，*annulimana* 種群，*bromeliae* 種群，*peruviana* 種群，*nannoptera* 種群，*carbonaria* 種群，*tumiditarsus* 種群，および *polychaeta* 種群などが含まれる．一方，*repleta* radiation で生じたものには，*repleta* 種群，*canalinea* 種群，*dreyfusi* 種群，*coffeata* 種群，および *mesophragmatica* 種群などが含まれる．

virilis-repleta 系統に関するこれまでの系統学研究は，これらの種群がある程度単系統的にまとまり，全体として Throckmorton（1975）の説と一致するような系統関係を示す．例えば，多くの分子系統学研究において，最初の放散で生じたとされる種群同士は必ずしも一つの単系統群を形成することなく，中でも *polychaeta* 種群は *virilis-repleta* 系統全体の根元から分岐する傾向にある．一方，*repleta* radiation で生じたとされる種群同士は比較的単系統的にまとまり，最初の放散で生じたとされる種群が，それらの側系統として位置付けられる（Tatarenkov and Ayala., 2001; Remsen and O'Grady, 2002; Robe et al., 2005, 2010; Wang et al., 2006; Da Lage et al., 2007; van der Linde and Houle, 2008; van der Linde et al., 2010）．これらの結果は，*virilis-repleta* 系統の祖先は旧大陸を起源とし，その後，一部の系統が新大陸で派生的に生じたとする Throckmorton（1975）の見解を支持する（Marcow and O'Grady, 2006）．

3.4.2.2 *immigrans-tripunctata* 系統

immigrans-tripunctata 系統は，*Drosophila* 亜属のうち，*virilis-repleta* 系統を除いた残りの種からなる．そのうち，*immigrans* 種群，*quinaria* 種群，*testacea* 種群，*bizonata* 種群，および *historio* 種群などは旧大陸に分布し，*tripunctata* 種群，*pallidipennis* 種群，*calloptera* 種群，*cardini* 種群，および *guarani* 種群などは新大陸での分布が認められる．Throckmorton（1975）によると，これらの種群は，*Hirtodrosophila* 属，*Mycodrosophila* 属，*Samoaia* 属，および *Zaprionus* 属などとともに，*immigrans-Hirtodrosophila* radiation と呼ばれる放散によって生じたとされる．しかし，分子系統解析による多くの研究では，上記の種群がある程度単系統的にまとまり，*Hirtodrosophila* 属や *Zprionus* 属とは別系統である可能性が示唆されることから，最近ではこれらをまとめて *immigrans-tripunctata* 系統として扱うようになった（Remsen and O'Grady, 2002; Yotoko et al., 2003; Robe et al., 2005, 2010; Markow and O'Grady, 2006; O'Grady and DeSalle, 2008; van der Linde et al., 2010）．ただし，いくつかの分子系統学研究では，これらの単系統性が必ずしも支持されない（Da Lage et al., 2007; Katoh et al., 2007）．

なお，*immigrans-tripunctata* 系統の中においては，*immigrans* 種群とそれ以外との間で最初に分岐することが，多くの研究から示唆されている（Remsen and O'Grady, 2002; Yotoko et al., 2003; Robe et al., 2005, 2010; van der Linde et al., 2010）．しかしながら，*immigrans* 種群を除いた残りの種群間の系統関係については未だに不明な点が多い（Pélandakis and Solignac, 1993; Remsen and O'Grady, 2002; Carrasco et al., 2003; Yotoko et al., 2003; Robe et al., 2005; Da Lage et al., 2007; Hatadani et al., 2009; van der Linde et al., 2010）．また，これらのうち，少なくとも *tripunctata* 種群と *guarani* 種群については，それぞれの種群で単系統にな

らないことが示唆されている（Carrasco et al., 2003; Yotoko et al., 2003; Robe et al., 2005; Da Lage et al., 2007; Hatadani et al., 2009; van der Linde et al., 2010）.

3.5 *Zaprionus* 属

Zaprionus 属はこれまで約 60 種が記載され，その多くがアフリカに分布し，いくつかの種が東南アジアやニューギニアに生息する．本属は *Phorticella* 属とともに，頭部から胸部背側にかけて数本の白い縦縞があることで特徴付けられる．本属の系統学的位置について，Throckmorton（1975）は *immigrans-Hirtodrosophila* radiation により生じた系統の一つであるとみなす一方，Grimaldi（1990）は，本属が *Phrticella* 属および *Samoaia* 属とともに *Zaprionus* genus group と呼ばれる単系統群を形成し，*Drosophila* 亜属を含む系統とはかけ離れた位置にあると示唆している．

これまでのいくつかの分子系統学研究では，本属が *Drosophila* 亜属を含むクレードの根元で分岐する樹形を示すことが多いが，その位置は安定せず，詳細については不明なままである（Pélandakis and Solignac, 1993; Kwiatowski et al., 1994, 1997; Kwiatowski and Ayala, 1999; Tatarenkov et al., 2001; Robe et al., 2005, 2010; Da Lage et al., 2007; Katoh et al., 2007; van der Linde and Houle, 2008; van der Linde et al., 2010）．また，最近の Yassin et al.（2010）の研究では，Grimaldi（1990）が唱えた *Zaprionus* genus group のうち，*Zaprionus* 属と *Phorticella* 属は多系統で，*Samoaia* 属は *immigrans* 種群の *D. quadrilineata* に近縁であることが示唆されている．

3.6 *Hirtodrosophila* 属

Hirtodrosophila 属はこれまで約 160 種が記載され，熱帯域を中心に分布が認められる．本属は以前，*Drosophila* 属の中の一亜属として位置付けられていたが（Sturtevant, 1942; Wheeler, 1981），Grimaldi（1990）はこれを属へと昇格させた．Throckmorton（1975）によると，本属は *immigrans-Hirtodrosophila* radiation の中の Old World *Hirtodrosophila* radiation によって生じたとみなされる．一方，Grimaldi（1990）は，本属が *Mycodrosophila* 属や *Zygothrica* 属とともに *Zygothrica* genus group と呼ばれる独立した系統群を形成し，*Drosophila* 属とは非常にかけ離れた系統であるとみなしている．

そして，これまでの分子系統学研究では，本属が *Drosophila* 亜属を含むクレードの根元で分岐する樹形を示すことが多いが，*Zaprionus* 属の場合と同様，その位置は不安定である（Kwiatowski et al., 1994, 1997; Kwiatowski and Ayala, 1999; Katoh et al., 2000, 2007; Tatarenkov et al., 2001; Robe et al., 2005; Da Lage et al., 2007; van der Linde and Houle, 2008; van der Linde et al., 2010）．ただし，少なくとも本属と *Mycodrosophila* 属との近縁性については，いくつかの分子系統学研究から示唆されており（Katoh et al., 2000; Remsen and O'Grady, 2002; Da Lage et al., 2007; van der Linde et al., 2010），この点については Throckmorton（1975）および Grimaldi（1990）の両者の説と一致する．また，本属のうち，*Hirtodrosophila duncani* については，同属の他のショウジョウバエとは明らかに異なる系統であることが示唆されている（van der Linde et al., 2010）.

3.7 *Scaptomyza* 属と Hawaiian *Drosophila*

Scaptomyza 属はこれまで 260 種以上が記載され，そのうち，約 160 種はハワイ固有で，残りは世界中に分布が認められる．一方，Hawaiian *Drosophila* は全てがハワイ固有で，これまで 400 種以上が記載されている．これらのショウジョウバエは顕著な適応放散の例の一つであること，そして，その進化的出来事の起こった絶対年代がハワイ列島を構成する島々の地質学的形成年代から推定できることから，進化機構を解明する上ですぐれた研究対象とされてきた（Throckmorton, 1966; Carson and Kaneshiro, 1976; Kaneshiro, 1980; Hardy and Kaneshiro, 1981; Carson and Yoon, 1982; Kambysellis et al., 1995; Tamura et al., 2004）.

Scaptomyza 属と Hawaiian *Drosophila* の系統学的位置については，従来，Throckmorton（1975）と Grimaldi（1990）の間で非常に異なる見解が得られている．まず，Throckmorton（1975）によると，この両者は近縁で，何れも *Drosophila* radiation の中の *immigrans-Hirtodrosophila* radiation で生じたとされる．一方，Grimaldi（1990）は，両者が姉妹群を形成せず，*Drosophila* 亜属を含む系統とはそれぞれ独立した系統であることを提唱し，それとともに，Hawaiian *Drosophila* には *Idiomyia* という属名を与えている．

しかしながら，分子系統学解析に基づくこれまでの研究は，*Scaptomyza* 属と Hawaiian *Drosophila* が姉妹群を形成するという点で Throckmorton（1975）を支持するが，このクレードが *virilis-repelta* 系統の姉妹群として位置付けられるという，新たな見解を示す点で注目される（Russo et al., 1995; Tamura et al., 1995; Katoh et al., 2000, 2007; Da Lage et al., 2007; O'Grady and DeSalle, 2008; van der Linde and Houle, 2008; Robe et al., 2010; van der Linde et al., 2010）.

また，*Scaptomyza* 属については，ハワイ固有種と世界中に分布する種が認められることから，本属がハワイ起源で一部が各大陸へ分散したという説と，本属は大陸起源で一部がハワイで適応放散したという説の二つが考えられる．この問題については現在もまだ決定的な解答が得られてないが，*Scaptomyza* 属が Hawaiian *Drosophila* と姉妹群を形成する点を考慮すると，どちらかと言うと前者を支持する傾向にある（Tamura et al.,

1995）．また，最近，O'Grady and DeSalle（2008）はmtDNAの約10 kbの塩基配列を用いて分子系統解析を行ない，その結果，ハワイ産の*Scaptomyza*属が大陸産の*Scaptomyza*属の側系統となる樹形が得られたことから，彼らも前者を支持している．

なお，いくつかの分子系統学研究では，Hawaiiam *Drosophila*種間におけるDNAの塩基置換率を基準とした分岐年代推定も行われている（Russo et al., 1995; Tamura et al., 2004）．これらの研究によると，*Scaptomyza*属とHawaiiam *Drosophila*のいくつかの亜属あるいは種群の分岐は，ハワイ列島が形成される以前に起こったという，興味深い推定がなされている．ちなみに，現在ハワイ列島が存在する地点には，ホットスポットと呼ばれるマントルの湧昇流が存在し，その上をプレートが北西に移動するに従ってハワイ列島が形成され，ミッドウェー諸島や天皇海山などはその名残であると考えられている（Carson and Clague, 1995）．従って，ハワイ産ショウジョウバエにおけるいくつかの祖先系統の分化は，ハワイ列島が出来る以前の，今では海に沈んだと思われる別の島で起こったかもしれない．

4．今後の展望

Grimaldiがショウジョウバエの系統に関する独自の仮説を提唱した1990年以降，これまで多くの分子系統学的解析に基づく研究が行なわれ，ショウジョウバエの系統に関する我々の理解は，この20年で飛躍的に向上した．しかしながら，その詳細についてはまだ不明な点も多く，今後もさらなる研究が必要である．例えば，*Hirtodrosophila*属，*Mycodrosophila*属，*Zaprionus*属，*Liodrosophila*属，および*Dorsilopha*亜属などの分類群は，何れも*Drosophila*亜属との近縁性を示すが，これらの具体的な系統学的位置については依然として不明なままである．また，*Drosophila*亜属の主な系統のうち，*immigrans-tripunctata*系統の内部の系統関係については未解決な部分が多く，今後の重要な課題として注目されている．そして，採集が容易でない，あるいは飼育が難しい等の理由により，分子系統解析に用いられてないショウジョウバエ種もまだまだ多数存在する．従って，ショウジョウバエ科の系統の全容を解明するためには，これらの種を含めた分子系統学的研究が必須である．

また，前項で述べたように，これまでの系統学研究から，少なくとも*Drosophila*属や*Drosophila*亜属については単系統群にならないことが確実と思われる．従って，将来的には，分子系統学的研究によって明らかにされた系統関係を反映するような，分類体系の再編が必要かもしれない（Dalton, 2009）．

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ギンガショウジョウバエ属（双翅目，ショウジョウバエ科）に関する系統分類研究の現状

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ギンガショウジョウバエ属は，頭部から胸部にかけて特徴的な銀白色縦条を持っており，そのため，同様に銀白色縦条を持つトゲアシショウジョウバエ属との分類の混乱が見られた．そこで，本稿では，まずギンガショウジョウバエ属の分類変遷について概観し，トゲアシショウジョウバエ属との分類の混乱の実態を明らかにした．次に，これまでのショウジョウバエ科のいくつかの系統仮説を紹介し，本属および，これまでに類縁が示唆された属との系統関係について考察した．さらに，銀白色縦条が非相同形質であることを明らかにした Yassin et al.（2010）の研究を紹介し，最後に，ギンガショウジョウバエ属の今後の研究の方向性を示した．

History and perspectives of taxonomic and phylogenetic research on the genus *Phorticella* Duda (Diptera, Drosophilidae)

Masanori Kondo[1]

Until the revisional work by Okada and Carson (1983), many taxonomists had confused the genus *Phorticella* with the genus *Zaprionus*, because of sharing the silvery or chalky white longitudinal stripes on their head and thorax. First, in this paper, I provided an overview of some taxonomic researches on *Phorticella* and their possible related genera. Second, I reviewed some hypotheses on the phylogeny of the family Drosophilidae and the previous work by Yassin et al. (2010) having shown that the silvery or chalky white longitudinal stripes on head and thorax are not homologous between the two genera. Finally, I presented some perspectives of researches on the genus *Phorticella*.

1. はじめに

ギンガショウジョウバエ属 *Phorticella* Duda（ショウジョウバエ科 Drosophilidae，ショウジョウバエ亜科 Drosophilinae）は，体長がおよそ 1.5–3.0 mm のハエで，本属の種の多くが頭部から胸部にかけて 1 対もしくは 2 対の銀白色縦条を持っている（図 1）．これまでに 2 亜属 11 種が記載されており，ギンガショウジョウバエ亜属（subgenus *Phorticella*）は旧北区，東洋区，オーストラリア区から 7 種，ニセギンガショウジョウバエ亜属（subgenus *Xenophorticella* Okada and Carson）は東洋区，エチオピア区から 4 種が知られている（Brake and Bächli, 2008）．頭部から胸部にある銀白色縦条は，ショウジョウバエ科のなかでも非常に特徴的な形質であることから，本属の分類に重要な形質と考えられてきた．しかし，エチオピア区～東洋区に分布しているトゲアシショウジョウバエ属 *Zaprionus* Coquillett（最近，そのうちの 1 種 *Zaprionus indianus* Gupta, 1970 は新熱帯区に侵入した；Vilela, 1999）もこの形質を持っていることから，両属の分類はこれまで非常に混乱しており，現在でも納得のいく分類体系は提示されていない．ショウジョウバエ科の分類における分子データの利用や，分子系統学的手法による系統解析は，飼育が容易なショウジョウバエ属 *Drosophila* Fallén で盛んに行われてきたが，近年，飼育が困難なグループにおいても分子系統学的研究が行われ始めてきた（Katoh et al., 2000; Zhao et al., 2009 など）．ギンガショウジョウバエ属やトゲアシショウジョウバエ属も分子データを用いた分類学的・系統学的研究が行われつつある．そこで，本稿では，まず本属の分類学的扱いの変遷について概観し，次に形態形質の分岐分類学的方法と DNA 分析によって得られた系統仮説を紹介し，本属とその近縁属と

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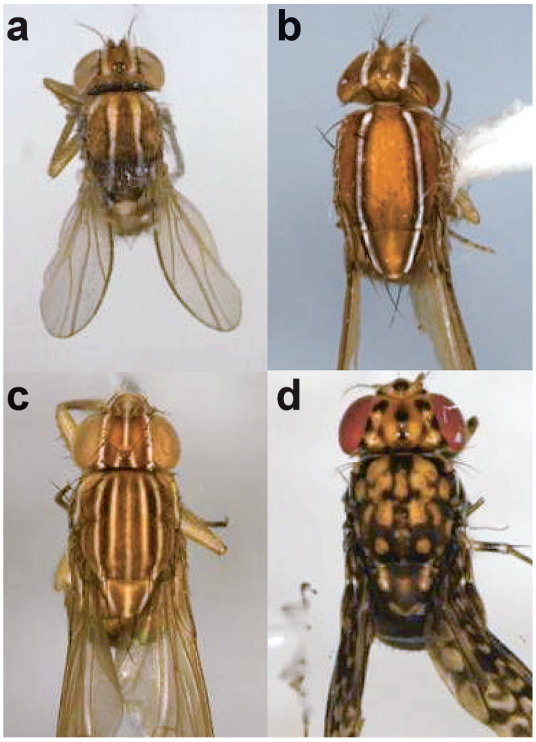

図 1：頭部および中胸の銀白色縦条．(a) *Phorticella* (*Phorticella*) *bistriata*, (b) *P*. (*Xenophorticella*)*flavipennis*, (c) *Zaprionus* (*Anaprionus*) *obscuricornis* (de Meijere, 1916), (d) *Samoaia leonensis* Wheeler and Kambysellis, 1966.

の系統関関係について議論する．

2．ギンガショウジョウバエ属の分類変遷

図 2 にギンガショウジョウバエ属の分類変遷について主なものを示す．

ギンガショウジョウバエ属は 1923 年に Duda によって，鼻瘤（carina）が低い，中眼縁剛毛（anterior reclinate orbital seta）が小さい，中胸背毛（acrostichal setulae）が 4 列，前脚腿節（fore femur）に瘤（tubercle）が無いといった形質に基づいて設立された．Sturtevant（1927）は，*Drosophila bistriata* de Meijere, 1911 をギンガショウジョウバエ属のタイプ種に指定すると共に，本属をトゲアシショウジョウバエ属の 1 亜属として扱った．Wilson et al.（1969）や Lin and Tseng（1972）は，*Drosophila immigrans* 種群について細胞学的研究，および分類学的研究をそれぞれ行い，それらの論文中で，現在はギンガショウジョウバエ属もしくは，トゲアシショウジョウバエ属とされている種，それぞれ 5 種，および 7 種の計 12 種を *D. lineosa* 種亜群として，ショウジョウバエ属に含めた．Wilson et al.（1969）は，中胸に白色縦条を持っている点で *D. lineosa* 種亜群とトゲアシショウジョウバエ属が類似していると述べている．しかし，アフリカ産のトゲアシショウジョウバエ属や中胸に銀白色模様を持つサモアイア属 *Samoaia* Malloch と，*D. lineosa* 種亜群は別属としてはっきりと区別できるとしている．Okada（1977）と Wheeler（1981）はそれぞれ東洋区，全世界についてのショウジョウバエ科の種のカタログを作成し，ギンガショウジョウバエ属を再び独立属として扱っている．しかしながら，これらの文献ではどの形質に基づきギンガショウジョウバエ属を独立属として判断したのか書かれていない．さらに Wheeler（1981）では，ギンガショウジョウバエ属とトゲアシショウジョウバエ属のどちらか所属が不明な種として 5 種が所属未決定のままリストされている．また，どちらのカタログにおいても，*P. singularis*（Duda, 1924）（銀白色縦条が胸部の途中で途切れている）がショウジョウバエ属として扱われていることから推測されるように，頭部から胸部にかけて存在する銀白色縦条が本属の分類に重要であると考えられており，同様に銀白色縦条を持つトゲアシショウジョウバエ属や *D. immigrans* 種群の *D. lineosa* 種亜群との分類が困難であった．この問題を解決するために，Okada and Carson（1983）はギンガショウジョウバエ属，トゲアシショウジョウバエ属，*D. immigrans* 種群

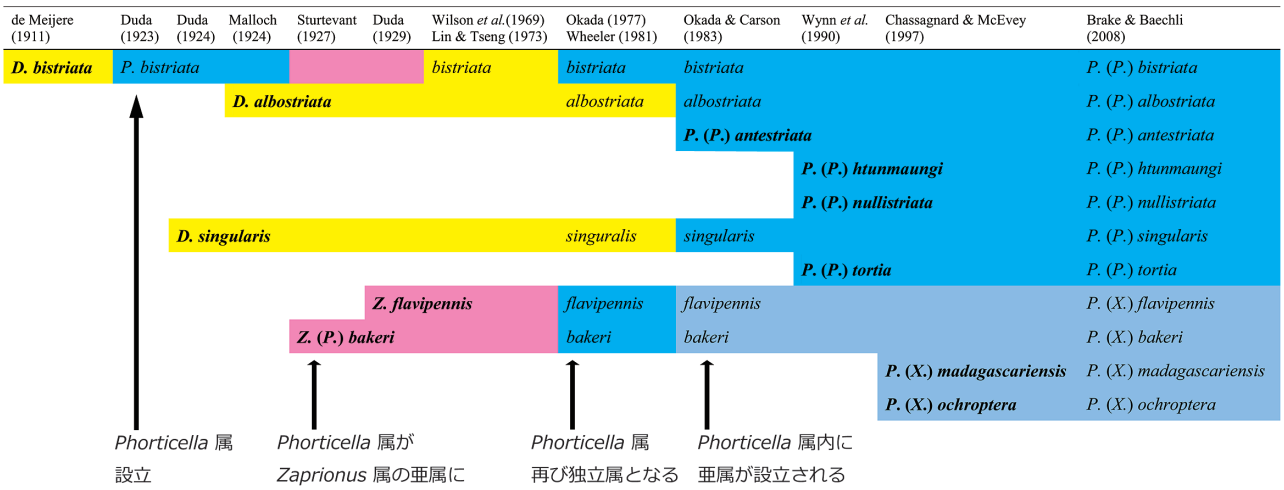

図 2：ギンガショウジョウバエ属の分類変遷．ギンガショウジョウバエ属 12 種について，記載されてからの属，もしくは亜属の分類の変遷を色分けして示してある．同物異名（junior synonym）などについてはここでは省略する．

の3群の，1）中眼縁剛毛の位置，2）中・後脚の跗節のくさび状剛毛の有無，3）雄の腹節背板第9節（epandrium，以下，雄第9背板）の形態，そして，4）雄の腹節腹板第9節（hypandrium，以下，雄第9腹板）中央部の剛毛数について検討を行った．ショウジョウバエ科の分類には雄交尾器の形態形質がよく用いられるが，本属において雄交尾器の形態形質による分類が行われたのはこの論文が初めてである．その結果，3群をはっきりと識別することができ，これらの形質を診断形質として，ギンガショウジョウバエ属とトゲアシショウジョウバエ属を再定義するとともに，前者をギンガショウジョウバエ亜属とニセギンガショウジョウバエ亜属に，後者をトゲアシショウジョウバエ亜属とトゲナシショウジョウバエ亜属（subgenus *Anaprionus*，原記載では*Aprionus*と命名されたが，後にItonididaeの*Aprionus* Kieffer, 1894の新参異物同名（junior homonym）であることがあることがわかり，改名された；Okada, 1990）のそれぞれ2亜属に分類した（表1）．しかし，亜属の分類において用いられた，頭部中央の銀白色縦条と，中・後脚のくさび状剛毛を持っているという形質はニセギンガショウジョウバエ亜属とトゲアシショウジョウバエ属に共通しており，亜属の分類形質を含めると，両属を定義する分類形質が混在していることになる．その後，Okada and Carson（1983）の定義に基づいて，Wynn et al.（1990）はギンガショウジョウバエ亜属の3新種を記載している．Chassagnard and McEvey（1997）はマダガスカルとスマトラ島からニセギンガショウジョウバエ亜属の2新種を記載している．Chassagnard and McEvey（1997）では，このとき記載された2新種と*P.*（*X.*）*bakeri*（Sturtevant, 1927）は，Okada and Carson（1983）においてニセギンガショウジョウバエ亜属を定義づける形質とされた頭部中央の銀白色縦条を持っていないことから，頭部中央の銀白色縦条は亜属を定義づける形質ではなく，*P.*（*X.*）*flavipennis* (Duda, 1929) が持つ種特有の形質ではないかと述べている．また，彼女らが記載した2新種のうち，*P.*（*X.*）*ochroptera*は第9腹板の亜中央棘毛が1対しかなく，Okada and Carson（1983）で定義されたギンガショウジョウバエ属の形質と一致しない．さらに，ニセギンガショウジョウバエ亜属とトゲナシショウジョウバエ亜属の定義にも混乱が見られる．ギンガショウジョウバエ属を定義する形態形質として，第9背板が伸長しないとされているが，ニセギンガショウジョウバエ属*P.*（*X.*）*flavipennis*の雄第9背板は，把握器（surstylus）と弱く癒合しており観察が困難であるが，よく見てみると雄第9背板はトゲアシショウジョウバエ属のように伸長していることが分かる．また，Wynn and Toda（1988）によって記載された，トゲナシショウジョウバエ亜属の*Z.*（*A.*）*aungsani*は〝2対〞の〝長い〞亜中央棘毛を持つと記されている．これは，Okada and Carson（1983）の定義によるとギンガショウジョウバエ属が持つ形態形質である．実際，雄交尾器のスケッチを見ると，ニセギンガショウジョウバエ亜属の雄交尾器形態と非常に類似していることがわかる．このように，従来のOkada and Carson（1983）による属の定義に当てはまらない種が見つかっていることから，Okada and Carson（1983）以降に記載された種も含めた分類学的再検討を行い，新しい属の定義を行う必要がある．

Okada and Carson（1983）は，ギンガショウジョウバエ亜属の雄交尾器とマメショウジョウバエ属*Scaptodrosophila* Dudaの雄交尾器の類似についても言及しており，その後のいくつかの研究によっても同様のことが指摘されている（Wynn et al., 1993; Chassagnard and McEvey, 1997）．マメショウジョウバエ属はDuda（1924）によって設立され，Wheeler（1949）によってショウジョウバエ属の1亜属となり，Grimaldi（1990）によって再び属へと昇格された経緯を持つ．これまでに世界で約300種が記載されており，ショウジョウバエ科の中でも種数の多い属の一つと言える（Brake and Bächli, 2008）．マメショウジョウバエ属は種数が多いことから，記載分類以外の分類学的研究はこれまでほとんど行われていないが，その形態的特徴の多様さから，おそらく多系統群となるのではないかと考えられる．マメショウジョウバエ属と本属との類縁関係については，交尾器の類似が指摘された他に研究例はないが，Bock（1978）は，現在ギンガショウジョウバエ属に含まれる

表1：Okada and Carson（1983）によって示されたギンガショウジョウバエ属，トゲアシショウジョウバエ属，*D. immigrans*種群を定義する形態形質．表中の属名，亜属名から〝ショウジョウバエ〞は省略している

形態形質	ギンガ属		トゲアシ属		*D. immigrans*種群
	ギンガ亜属	ニセギンガ亜属	トゲアシ亜属	トゲナシ亜属	
中眼縁剛毛の長さ	小さい		よく発達		発達
後眼縁剛毛の位置	前眼縁剛毛により近い		前眼縁剛毛と内頭頂剛毛の中央		前眼縁剛毛により近い
雄第9背板の形態	伸長していない		伸長している		
雄第9腹板の亜中央棘毛の本数／長さ	2対／長い		1対／普通	1対 or 2対／短い	
頭部中央の銀白色縦条	なし	あり	あり		
中・後脚のくさび状剛毛	なし	あり	あり		
中胸の銀白色縦条数			4本	5本 or 7本	

P. (*P.*) *albostriata* (Malloch, 1924) の雄交尾器を観察し，マメショウジョウバエ属（当時はショウジョウバエ属マメショウジョウバエ亜属）に含まれるのではないかと考察している．筆者らの研究でも，現在マメショウジョウバエ属とされている種の中に，その形態学的特徴からギンガショウジョウバエ属とした方がよいと思われる種が含まれていることが示唆されており，今後マメショウジョウバエ属とギンガショウジョウバエ属との関係についてもさらなる研究が必要であろう．

3．ギンガショウジョウバエ属の系統学的研究

ギンガショウジョウバエ属はこれまでに述べたとおり，トゲアシショウジョウバエ属や *D. immigrans* 種群と同属として扱われたり，また，ギンガショウジョウバエ亜属とマメショウジョウバエ属の雄交尾器形態の類似性が言及されたりしてきたが，これらの属間の系統関係についての研究はほとんど行われていない．そこで，本稿ではショウジョウバエ科のいくつかの代表的な系統仮説について，これまでに本属との類縁性が示唆されたグループの系統的位置や系統関係に的を絞って紹介することで，ショウジョウバエ科における本属の系統的位置について考察したい．

表 2 に，ショウジョウバエ科の系統仮説の代表的なものについて，提唱者，用いた分類群の数，系統推定に用いた形質を示す．

3.1 形態形質に基づく系統仮説

形態形質に基づく系統仮説の代表的なものとして，Throckmorton（1975）仮説があり，その対立仮説として Grimaldi（1990）が挙げられる．Throckmorton（1975）の系統仮説は，卵や蛹，雌雄成虫の内部生殖器官などの形態形質の比較研究に基づき系統推定が行われている．彼の系統推定では，ショウジョウバエ科の系統進化の過程において，いくつかの放散のイベント（Radiation）が生じ，それぞれの Radiation で様々な系統群が分化してきたと考えられている．彼の系統仮説に，ギンガショウジョウバエ属は含まれていない．本属と分類の混乱が見られたトゲアシショウジョウバエ属や *D. immigrans* 種群はサモアイア属や *D. quinaria* 種群などの一部のショウジョウバエ属と共に，immigrans-Hirtodrosophila Radiation から分化したと考えられている．一方で，ギンガショウジョウバエ亜属との交尾器の類似が示唆されたマメショウジョウバエ属は，ショウジョウバエ亜科の最初の放散である Scaptodrosophila Radiation から分化したと考えられ，トゲアシショウジョウバエ属や *D. immigrans* 種群とは，系統的に離れた関係にあると言える．

Grimaldi（1990）はショウジョウバエ科のほぼすべての属を網羅する，非常に多くの属，亜属について，成虫の外部形態形質に基づき系統解析を行った．その結果，ギンガショウジョウバエ属はトゲアシショウジョウバエ属，サモアイア属と共にクレードを形成した．このクレードは，中胸に 1 対以上の銀白色縦条を持つ，中脚・後脚にくさび状剛毛を持つ，などの診断形質に基づいて，トゲアシショウジョウバエ属群として定義された（図 3）．しかし，Grimaldi（1990）の解析でギンガショウジョウバエ属の代表として選定された *P. argentstriata*（Bock, 1966）は，Okada and Carson（1983）の分類学的改訂以来，トゲアシショウジョウバエ属の種

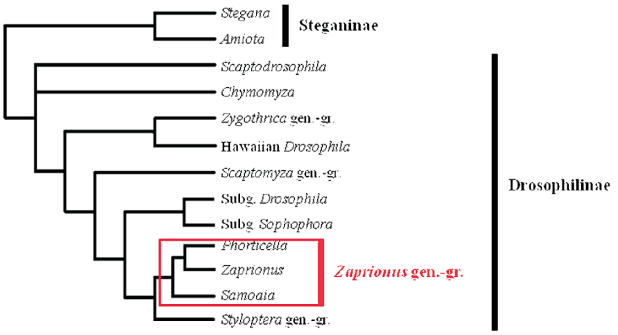

図 3：Grimaldi（1990）によるショウジョウバエ科の系統仮説．本文と関係のない属については多少省略する．

表 2：ショウジョウバエ科の様々な系統仮説．代表的なものについて，提唱者，分類群の数，推定に用いた形質について示してある．その他の系統仮説については van der Linde and Houle（2008）を参照されたい

提唱者	分類群	系統推定に用いた形質
ショウジョウバエ科全般		
Throckmorton（1975）	65 群（属，種群など）	**形態形質**（卵，蛹，成虫内部形態など）
Grimaldi（1990）	50 属 127 種	**形態形質**（雌雄成虫外部形態）
Remsen and O'Grady（2002）	20 属 46 種	**DNA データと形態形質の結合系統樹**（形態，*16S, 28S, Adh, COII, Sod*）
van der Linde and Houle（2008）	9 属 297 種	**スーパーツリー法による系統樹**（127 系統樹を基に作成）
van der Linde et al.（2010）	12 属 126 種	**DNA データ**（Supermatrix）（*Adh, Amyrel, per, Ddc, Sod, ypl, 28Sdl, 28Sd2, 28Sd8, COI, COII, COIII, 16S*）
ギンガショウジョウバエ属が含まれるもの		
Yassin et al.（2010）	6 属 25 種	**DNA データ**（*Amyrel, COII*）

とされている．したがって，Grimaldi（1990）の解析にはギンガショウジョウバエ属が含まれていなかったことになる．なお，*Zaprionus argentostriatus*（Bock, 1966）は，Wynn and Toda（1988）によって *Zaprionus bogoriensis* Mainx, 1958 の新参同物異名（junior synonym）とされた．

3.2 DNA データに基づく系統仮説

DNA データを用いたショウジョウバエ科の系統推定は，多くの研究者によって行われている．DNA データを用いた系統解析の中には，DNA データと形態形質データを組み合わせた解析や（Remsen and O'Grady, 2002），過去に発表された 127 の系統樹をスーパーツリー法によって解析したものまで（van der Linde and Houle, 2008），様々な DNA 領域や，解析方法によってショウジョウバエ科の系統推定が試みられている．これまでの研究については van der Linde and Houle（2008）の表にわかりやすくまとめられているので，そちらを参照されたい．ここでは，van der Linde et al.（2010）によって提唱されたショウジョウバエ科の系統仮説について紹介したい．van der Linde et al.（2010）は 12 属 176 種について最大 13 の異なる遺伝子座の DNA 配列を用いて系統解析を行った．図 4 は van der Linde et al.（2010）の最尤法によって得られた系統樹を要約したものである．ギンガショウジョウバエ属とサモアイア属は解析に含められておらず，トゲアシショウジョウバエ属群の系統関係について議論することはできない．彼女らの系統仮説では，トゲアシショウジョウバエ属は *D. immigrans* 種群や *Hirtodrosophila* 属，*Mycodrosophila* 属とクレードを形成しており，これは Throckmorton（1975）が示唆した immigrans-Hirtodrosohila Radiation とよく一致している．さらに，ギンガショウジョウバエ亜属と雄交尾器の類似性が示唆されたマメショウジョウバエ属は，他のショウジョウバエ亜科と姉妹群関係となる系統と *Chymomyza* 属と姉妹群関係となる系統（*rufifrons* 種群＋*victoria* 種群）の 2 つの異なる系統から構成される非単系統群であることが示唆された．

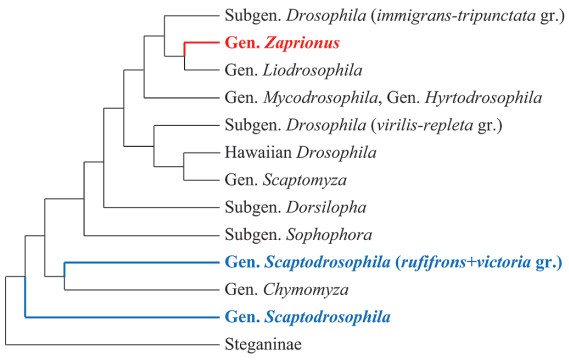

図 4：van der Linde et al.（2010）によるショウジョウバエ科の系統仮説．本文と関係のない属については多少省略する．

3.3 ギンガショウジョウバエ属は単系統群か？

これまでのいずれの系統仮説においても，ギンガショウジョウバエ属は解析に含まれておらず，またトゲアシショウジョウバエ属群を構成する 3 属を含む解析も行われていないため，ギンガショウジョウバエ属の系統的位置や，トゲアシショウジョウバエ属群の単系統性を議論することはできなかった．この状況を解決するために，Yassin et al.（2010）はトゲアシショウジョウバエ属群の単系統性を検証することを目的としてギンガショウジョウバエ属 2 亜属 4 種を含む，6 属 25 種について *Amyrel* 領域と *COII* 領域の DNA 配列に基づき系統解析を行った．その結果，トゲアシショウジョウバエ属群を構成する 3 属はすべて異なるクレードに含まれ，トゲアシショウジョウバエ属群は非単系統群であることが示唆された．さらに，ギンガショウジョウバエ亜属は他のショウジョウバエ亜科すべてと姉妹群関係になり，一方，ニセギンガショウジョウバエ亜属はトゲナシショウジョウバエ亜属の一部とクレードを形成し，ギンガショウジョウバエ属の単系統性を支持しないとする非常に興味深い結果が得られた．Yassin et al.（2010）はさらに，トゲアシショウジョウバエ属群を定義していた中胸の銀白色縦条の形質に注目し，電子顕微鏡を用いて観察を行ったところ，トゲアシショウジョウバエ亜属とニセギンガショウジョウバエ亜属の銀白色縦条は，微小毛（microtrichia）が密集して生えることによる構造色であり，それに対してギンガショウジョウバエ亜属やトゲナシショウジョウバエ亜属の銀白色縦条には，密集して生える微小毛はなく，色素の欠損もしくは白色色素の沈着によって生じたものであることが明らかとなったことから，長年，ギンガショウジョウバエ属とトゲアシショウジョウバエ属の分類の混乱を招いていた銀白色縦条は，異なる起源に由来する非相同な形質であることが示唆された．

4．ギンガショウジョウバエ属の研究の今後

Yassin et al.（2010）の研究により，1）ギンガショウジョウバエ属が非単系統群であること，2）銀白色縦条は非相同形質であり，属間および亜属間の分類に用いることができないことが示唆された．従来のギンガショウジョウバエ属の単系統性が支持されなかったことから，ニセギンガショウジョウバエ亜属を独立属とするのか，それともトゲアシショウジョウバエ属とするのかなどの分類学的扱いを含め，ギンガショウジョウバエ属の分類学的再検討が必要である．同時に，系統学的解析も行い，非単系統群であることが示唆されたマメショウジョウバエ属との系統関係についてもさらに検討する必要がある．筆者のこれまでの研究では，van der Linde

et al.（2010）のマメショウジョウバエ属の 2 系統のうち，*rufifrons* 種群＋*victoria* 種群は，ギンガショウジョウバエ亜属といくつかの交尾器形態の形質状態を共有しており，両群が系統学的に近縁な関係にあるのではないかということが示唆されている．今後，ギンガショウジョウバエ属と，マメショウジョウバエ属の種数を増やした系統解析を行うことで，両属の単系統性や系統関係についての新しい系統仮説および，共有派生形質に基づく新分類体系を提示できるのではないかと思われる．

最後に，銀白色縦条についても，今後の研究に期待されることを記しておきたい．Yassin et al.（2010）によって，銀白色縦条が複数の系統群で進化してきた非相同形質であることが示唆され，ギンガショウジョウバエ亜属は微小毛による銀白色縦条を持たないとされていた．しかし，最近の筆者らの研究により，ギンガショウジョウバエ亜属内においても，微小毛の構造色による銀白色縦条を持つ種の存在が明らかになってきた．なぜ，微小毛の獲得（もしくは消失）が，複数の系統群で独立に行われたのか，その発生メカニズムはどのようになっているのか？　また，銀白色縦条はギンガショウジョウバエ属やトゲアシショウジョウバエ属にとってどのような進化的・生態的意義を持っているのか？　今後，様々な分野において更なる研究が期待されるショウジョウバエである．

謝辞

本稿は 2010 年 9 月に北海道大学で開催された，ワークショップ「ショウジョウバエ研究のいまとこれから――特に，キイロショウジョウバエ以外の研究に関して――」での発表の内容をもとに執筆された．ワークショップの世話人である首都大学東京の田村浩一郎教授，北海道大学の戸田正憲教授，愛媛大学の和多田正義教授には，発表の機会を与えていただきました．さらに，戸田正憲教授には，本誌への投稿を勧めていただき，また，草稿に対し丁寧な添削をしていただきました．ワークショップでは，多くの方々から有益な助言をいただきました．以上の方々に厚く御礼申し上げます．加えて，日頃，研究指導をして頂いている九州大学の荒谷邦雄教授にも記して感謝申し上げたい．なお，本稿の執筆にあたり，文部科学省グローバル COE プログラム（自然共生社会を拓くアジア保全生態学）の支援を受けた．

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Molecular phylogeny of the *cristata* species group of the genus *Colocasiomyia* (Diptera: Drosophilidae)

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Flies of the *Colocasiomyia cristata* species group depend their life cycles on specific host plants of the family Araceae and play important roles as species-specific pollinators in return. A pair of 'stamenicolous' and 'pistillicolous' species cohabit in the same inflorescence and such pairs have been reported from different host species and different geographical regions. To understand the evolution of host selection and cohabitation and the divergence between stamenicolous and pistillicolous species in the *C. cristata* group, we conducted molecular phylogenetic analyses. The mitochondrial *ND2* sequences (maximum 858 base pairs) of 27 OTUs were analysed. Phylogenetic relationships reconstructed by the neighbour-joining, maximum parsimony and maximum likelihood methods were summarised in a consensus network. The resolution in the inferred phylogeny was higher than those in the previous cladistic studies based on the morphological characteristics. We recognised three main clades: the *C. colocasiae-alocasiae*, *C. diconica-xenalocasiae* and *C. cristata-sulawesiana* clades. Comparisons of ecological traits among species on their phylogenetic relationships revealed that the origins of cohabitation and stamenicolous and pistillicolous breeding habits are more complicated than thought in the previous hypothesis. Since the number of the species is limited in this study, more biogeographic information and comparative ecological studies based on reliable phylogenetic trees with comprehensive taxon sampling of both *Colocasiomyia* flies and their host plants will be necessary.

1. Introduction

The genus *Colocasiomyia* de Meijere, 1914 (Diptera: Drosophilidae) comprises 26 described species and approximately 46 undescribed species distributed in the Oriental and Papuan regions (Sultana et al., 2006; Toda et al., unpubl. data) and is classified into five species groups (Okada, 1990; Sultana et al., 2002; Sultana et al., 2006; Yafuso et al., 2008). The members of each species group visit a specific taxonomical group or groups of host plants in the families Araceae, Arecaceae and Magnoliaceae (Sultana et al., 2006) (Fig. 1). Flies of the *C. cristata* species group exhibit especially high host specificity: each species usually utilises only one or two host plant species.

In addition to the host specificity, particular breeding habits and larval development in their host inflorescences and infructescences were reported for certain *Colocasiomyia* species. It is sharing of the same inflorescence by a pair of *Colocasiomyia* species with microallopatric breeding niche separation: one species, referred to as "pistillicolous species", mainly utilises the lower pistillate region (female-inflorescence) of spadix for oviposition and larval development, whereas the other, referred to as "stamenicolous species", the upper staminate region (male-inflorescence). This phenomenon was first

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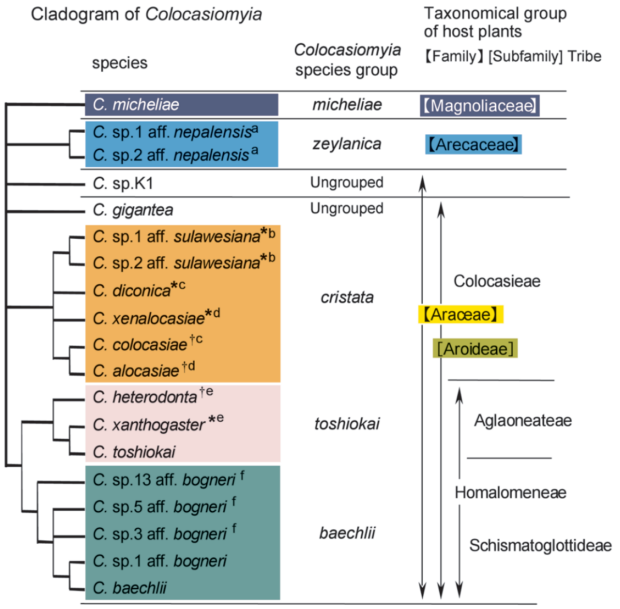

Figure 1：A hypothesis on *Colocasiomyia* phylogeny and species groups corresponding to the taxonomical groups of their host plants [after Sultana et al. (2006) with modification]. The cladogram was obtained based on 62 morphological characters. The members of the *Colocasiomyia cristata* species group visit inflorescences of the tribe Colocasieae (sensu Mayo et al., 1997, Araceae), the *C. baechlii* species group to the tribe Schismatoglottideae (sensu Mayo et al., 1997, Araceae), the *C. crassipes* species group to *Michelia* species (Magnoliaceae), the *C. toshiokai* species group to the tribe Homalomeneae (sensu Mayo et al., 1997, Araceae) and the *C. zeylanica* species group to *Pinanga* species (Arecaceae). †Stamenicolous species; * pistillicolous species; each alphabet (a-f) indicates cohabiting species in the same host plant.

regarded as "synhospitalic pairs" or "synhospitalism" (Eichler, 1966) by Okada (1980) for *Colocasiomyia* flies, but is referred to as "cohabitation" in the present study.

Such cohabiting traits have been reported in different host plants, from different geographic areas and on different species groups of *Colocasiomyia* flies. For example, some species pairs of the *C. cristata* group have been reported to breed together on inflorescences of the genera *Colocasia* and *Alocasia* (Carson and Okada, 1980; Honda-Yafuso, 1983; Toda and Okada, 1983; Okada and Yafuso, 1989). *Colocasiomyia stamenicola* (Carson & Okada, 1980) and *C. pistilicola* (Carson & Okada, 1980) have been recorded from inflorescences of *Colocasia esculenta* (L.) Schott (Carson and Okada, 1980), and *C. alocasiae* (Okada, 1975) and *C. xenalocasiae* (Okada, 1980) from *Alocasia odora* (Roxb.) K. Koch (Honda-Yafuso, 1983; Yafuso, 1994).

In some species, and presumably in most species, *Colocasiomyia* flies seem to depend on inflorescences and infructescences of host plants almost throughout their entire life cycles (Takenaka, 2006). In return, some *Colocasiomyia* species have been confirmed to play important roles as species-specific pollinators for their host plants (Carson and Okada, 1980; Kramadibrata and Hambali, 1983; Yafuso, 1993; Mori and Okada, 2001; Miyake and Yafuso, 2003; Takenaka et al., 2006). *Colocasiomyia* flies and their host plants may have co-evolved through such intimate relationships. Further, these pollination mutualisms may have increased species-diversity of both *Colocasiomyia* flies and their host plants.

During the last decade, a considerable number of new species have been found in the genus *Colocasiomyia* (Sultana et al., 2002; Sultana et al., 2006; Toda and Lakim, in press; Toda et al., unpubl. data). These discoveries lead us to a new phase of study on the taxonomy and ecology of this genus, because the observed variation in ecological traits of some new species cannot be explained in accordance with so far proposed hypotheses for cohabitation of stamenicolous and pistillicolous species: in some systems one fly species monopolizes the host inflorescences (Takenaka et al., 2006), while others may cohabit with up to seven other species (Toda and Lakim, in press).

Okada (1980, 1986b, 1988, 1990) carried out phenetic analyses and proposed the classification of species groups within the genus *Colocasiomyia*. Grimaldi (1991) was the first to examine phylogenetic relationships among species of this genus by a cladistic analysis using 22 morphological characters. Sultana et al. (2006) conducted another cladistic analysis for 21 *Colocasiomyia* species based on 62 morphological characters and revised the classification of species groups within this genus (Fig. 1). The cladograms resulting from these two analyses were consistent in supporting the monophyly of the *C. zeylanica* and *C. toshiokai* species groups but incongruent with each other at many points. The *C. cristata* group was suggested as a paraphyletic group in Grimaldi (1991). Sultana et al. (2006) recovered its monophyly, supporting the proposition of this species group by Okada (1990), but remained phylogenetic relationships among and within species groups largely unresolved (Fig. 1). As to the relationships within the *C. cristata* group, stamenicolous species such as *C. colocasiae* (Duda, 1924), *C. alocasiae* and *C. stamenicola* formed a clade in both cladograms, but the phylogenetic positions of

pistillicolous species such as *C. xenalocasiae*, *C. diconica* (Toda & Okada, 1983) and *C. sulawesiana* Okada & Yafuso, 1989 were unstable or remained unresolved.

To understand the evolutionary processes of mutualism between *Colocasiomyia* flies and their host plants and cohabitation between stamenicolous and pistillicolous species, we must know precise phylogenetic relationships among the species. In this study, we aimed to resolve the phylogenetic relationships within the *C. cristata* group based on a molecular phylogenetic analysis using the mitochondrial NADH dehydrogenase subunit 2 (*ND2*) gene sequences and to consider the evolutionary origins of the breeding habits, cohabitation and host selection in this group.

2. Materials and methods

Twenty-six operational taxonomic units (OTUs) were selected as the ingroup (Table 1). On the basis of the research by Sultana et al. (2006), we included the DNA sequences of *Scaptodrosophila lebanonensis* (Wheeler, 1949) (the GenBank accession number HQ110598), *Drosophila immigrans* Sturtevant, 1921 (HQ110577), *D. virilis* Sturtevant, 1916 (HQ110586) and *D. funebris* (Fabricius, 1787) (HQ110575) as the outgroup. The sequences of *C. alocasiae* and *C. xenalocasiae* from Okinawa were provided by R. Segawa of Tokyo Metropolitan University. Adults of other *Colocasiomyia* flies were collected in the field between 2003 and 2007 and were preserved in 99.5% ethanol until DNA extraction. DNA was extracted from one adult fly by the method of Boom et al. (1990) with some modification (Kobayashi et al., 2009). The *ND2* fragment was amplified by the polymerase chain reaction (PCR) technique. Nucleotide sequences for the primers were as follows: *ND2* HEAD, 5'-AAGCTACTGGGTTCATACC-3' (tRNA-Trp); *ND2* TAIL, 5'-ATATTTACAGCTTTGAAGG-3' (tRNA-Met). PCR was done in 25 μL reaction volume with mixture contained 2 m*M* Tris-HCl pH 8.0, 10 m*M* KCl, 0.01 m*M* EDTA, 0.1 m*M* DTT, 0.05% Tween 20, 0.05% Nonidet P-40, 0.2 m*M* of each dNTP, 5–10 μ*M* of each primer, approximately 0.5 μg template DNA and 0.65 units Blend Taq polymerase™ (TOYOBO, Japan). PCR amplification were performed for 35 cycles using ATC 201 thermal cycler (Apollo, Belgium) under the following parameters: 94°C for 30 s, 52°C for 30 s, 72°C for 1 min, except for the first cycle of 94°C for 3 min and the last cycle of 72°C for 10 min. DNA sequencing reaction was carried out using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, USA) and the nucleotide sequences were determined using ABI PRISM® 3100-Avant Genetic Analyzer according to the protocol supplied by the manufacturer. DNA sequences were analyzed by Sequencing Analysis Software v3.7 (Life Technologies) and MEGA software version 5 beta 7 (Tamura et al., 2007). Finally, we retrieved maximum 858 base pairs of the coding region for each resulting sequence (DDBJ Accession numbers AB609013－AB609038, Table 1). We aligned the sequences using ClustalW program with codon model implemented in MEGA 5 with gap opening penalty 10 and gap extension penalty 0.1 for pairwise alignment and gap opening penalty 10 and gap extension penalty 0.2 for multiple alignment following the default setting of the program.

We performed the neighbour-joining (NJ) and maximum parsimony (MP) methods using MEGA software. All codon positions were included, but all indels and ambiguous sites were treated as missing data. In the NJ phylogenetic reconstruction, the evolutionary distances were computed using the Tamura-Nei (1993) model and pairwise deletion option. In the MP phylogenetic reconstruction, the most parsimonious trees were obtained using the close-neighbour-interchange (CNI) algorithm at search level 3, in which the initial trees were obtained by random addition of sequences (50 replicates). There were 858 sites in the final dataset, of which 337 were parsimony informative. The strict consensus tree was generated from the two most parsimonious trees. We also performed the maximum likelihood (ML) phylogenetic reconstruction. The KAKUSAN software (Tanabe, 2007) version 4 was used to determine the appropriate model of DNA substitution; as a result, CodonProportional with TVM+Gamma model was selected by the corrected Akaike Information Criterion (AICc4). ML analyses were performed using Treefinder (Jobb, 2008) with the 100 iterations of the likelihood ratchet implemented in Phylogears software version 2 (Tanabe, 2010).

To assess the branch confidence on the NJ, MP and ML trees, the bootstrap test (Felsenstein, 1985) was performed with 1,000 replicates. The resultant trees were rooted using the outgroup. To help the visualisation of conflicting or ambiguous phylogenetic

Table 1：Operational taxonomic units (OTUs) used for the molecular phylogenetic analyses

	Subfamily	Genus	Subgenus	Species group	Species-subgroup	Species	ID	Locality	Accession No.
1	Drosophilinae	*Scaptodrosophila*				*lebanonensis*			HQ110598
2		*Drosophila*	(*Drosophila*)	*immigrans*		*immigrans*			HQ110577
3			(*Drosophila*)	*virilis*		*virilis*			HQ110586
4			(*Drosophila*)	*funebris*		*funebris*			HQ110575
5		*Colocasiomyia*		ungrouped		*gigantea*	K2-19	Bogor Botanical Garden, West Java, Indonesia	AB609013
6						*gigantea*	K2-20	Bogor Botanical Garden, West Java, Indonesia	AB609014
7						sp.X1	GaoG1	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609015
8				*cristata*	*colocasiae*	*colocasiae*	K7-2	Situ Tengah, Bogor, West Java, Indonesia	AB609016
9					*colocasiae*	*colocasiae*	K4-8	Kg. Apin Apin, Keningau, Sabah, Borneo, Malaysia	AB609017
10					*colocasiae*	*colocasiae*	K7-3	Kinabalu Park Head Quarter, Sabah, Borneo, Malaysia	AB609018
11					*colocasiae*	*iskandari*	2006v04-26	Kerinci, Sumatra, Indonesia	AB609019
12					*colocasiae*	sp.1 aff. *iskandari*	2006v04-27	Probolinggo, Madakaripura, East Java, Indonesia	AB609020
13					*colocasiae*	*steudnerae*	2006v04-17	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609021
14					*colocasiae*	*alocasiae*	Segawa	Okinawa, Japan	AB609022
15					*colocasiae*	*alocasiae*	K8-2	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609023
16						sp. aff. *stamenicola*	K8-12	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609024
17						sp. aff. *stamenicola*	2006v04-18	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609025
18						*xenalocasiae*	Segawa	Okinawa, Japan	AB609026
19						*xenalocasiae*	2006v04-16	Xishuangbanna Tropical Botanical Garden, Yunnan, China	AB609027
20						*diconica*	K8-6	Situ Tengah, Bogor, West Java, Indonesia	AB609028
21						*diconica*	K8-3	Kg. Apin Apin, Keningau, Sabah, Borneo, Malaysia	AB609029
22						*diconica*	K8-7	Padan-Sukalami, West Sumatra, Indonesia	AB609030
23						sp.1 aff. *diconica*	K8-4	Bogor Botanical Garden, West Java, Indonesia	AB609031
24						sp.1 aff. *diconica*	K8-8	Madakaripura, East Java, Indonesia	AB609032
25						*cristata*	K2-6	Bogor Botanical Garden, West Java, Indonesia	AB609033
26						*cristata*	K2-15	Bogor Botanical Garden, West Java, Indonesia	AB609034
27						*cristata*	K4-2	Ciamis, West Java, Indonesia	AB609035
28						*sulawesiana*	K4-3	Enrekang, South Sulawesi, Indonesia	AB609036
29						sp.1 aff. *sulawesiana*	K4-4	Kota Kinabalu, Sabah, Borneo, Malaysia	AB609037
30						sp.2 aff. *sulawesiana*	K7-1	Kota Kinabalu, Sabah, Borneo, Malaysia	AB609038

signals among the NJ, MP and ML trees, a consensus network (Holland et al., 2004) was computed using the SplitsTree4 package (Huson and Bryant, 2006) without edge weights (Holland et al., 2006). The consensus network was modified to a species tree (network) and compared with information on cohabiting pairs, host plant and breeding characteristics.

3. Results

All conspecific OTUs formed monophyletic clades except for *C. alocasia* in the ML tree (Fig. 2C). The branching patterns of the NJ, MP and ML trees mostly agreed with each other except five branches that were associated with relatively lower bootstrap values (Figs 2A–C). Such topologies with signals of phylogenetic uncertainty were represented by box structures with parallel edges in the consensus network, which was computed from the NJ tree, the two most parsimonious trees and the ML trees as input (Fig. 2D). One of the five box structures was located at the clade connecting *C. sulawesiana*, *C.* sp.1 aff.

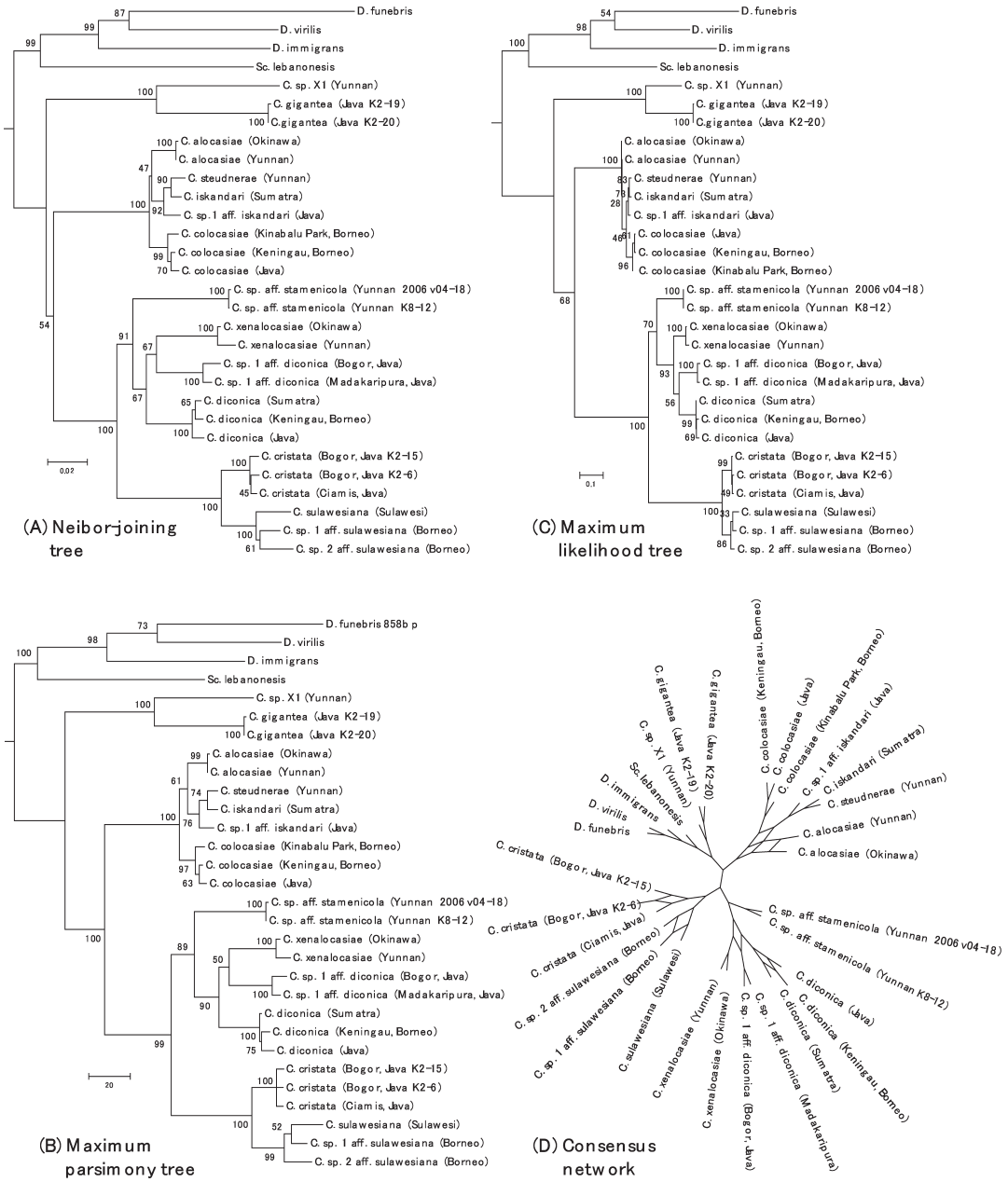

Figure 2 : The phylogenetic relationships among OTUs in the *Colocasiomyia cristata* species group and related taxa. (A) The neighbour-joining tree drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. (B) The consensus tree of two most parsimonious trees; branches without consensus are collapsed; except for the multifurcated branches, the tree is drawn to scale with branch lengths calculated using the average pathway method in the units of the number of changes over the whole sequence. (C) The maximum likelihood tree. (D) The consensus network. See the text for more details.

sulawesiana and *C*. sp.2 aff. *sulawesiana* and the other remaining OTUs. This structure simultaneously represents two different topologies: (*C. sulawesiana*, (*C*. sp.1 aff. *sulawesiana*, *C*. sp.2 aff. *sulawesiana*)) in the NJ tree and ((*C. sulawesiana*, *C*. sp.1 aff. *sulawesiana*), *C*. sp.2 aff. *sulawesiana*) in the MP and ML trees. Likewise, two other box structures were located between external branches in each conspecific clade of *C. diconica* and *C. cristata* de Meijere, 1914. For the remaining boxes, each box was located at a node that connects three different species or clades: (*C. diconica*, *C*. sp.1 aff. *diconica* and *C. xenalocasiae*) and (*C. colocasiae*, *C. alocasiae* and (*C. steudnerae* Takenaka & Toda, in Takenaka et al., 2006, *C. iskandari* (Okada, 1986) and *C*. sp.1 aff. *iskandari*)) . The *C. colocasiae-alocasia* clade exhibited a reticulated structure because of the paraphyletic topology of *C. alocasiae* in the ML tree.

We redrew the consensus network (Fig. 2D) as a modified rooted species network (i.e. species tree) for the ingroup (Fig. 3). The clade consisting of *C. gigantea* (Okada, 1987) and *C*. sp.X1 was placed as the sister to the *C. cristata* group. The *C. cristata* group was first divided into two clades associated with high bootstrap values (99–100%, Figs 3A–C), one comprising *C. colocasiae, C. alocasiae, C. steudnerae, C. iskandari* and *C*. sp.1 aff. *iskandari* and the other comprising the remaining species. The latter further diverged into two clades associated with moderate to high bootstrap values (70–100%): (*C*. sp. aff. *stamenicola*, *C. xenalocasiae*, *C. diconica* and *C*. sp.1 aff. *diconica*) and (*C. cristata*, *C. sulawesiana*, *C*. sp.1 aff. *sulawesiana* and *C*. sp.2 aff. *sulawesiana*). Within each clade, *C*. sp. aff. *stamenicola* first diverged from (*C. xenalocasiae*, *C. diconica* and *C*. sp.1 aff. *diconica*) and *C. cristata* first diverged from (*C. sulawesiana*, *C*. sp.1 aff. *sulawesiana* and *C*. sp.2 aff. *sulawesiana*).

4. Discussion

4.1 Phylogenetic relationships among species

In the cladistic analysis of Sultana et al. (2006), *C. gigantea* was not assigned to any species group in the genus *Colocasiomyia*. The results of the present study suggested that *C. gigantea* formed a monotypic lineage together with *C*. sp.X1, a taxonomically undescribed species, which had not been discovered at the time of the study by Sultana et al. (2006). However, the phylogenetic position of the clade (*C. gigantea*+*C*. sp.X1) within the genus remained unresolved because no species group other than the *C. cristata* group was included in our phylogenetic analyses.

Consistent results between Sultana et al. (2006) and the present study were that both of the *C. cristata-sulawesiana* clade and the *C. colocasiae-alocasiae* clade were recognized within the *C. cristata* group, although Sultana et al. (2006) included only *C. colocasiae and C. alocasiae* for the former clade and only *C*. sp.1 aff. *sulawesiana* and *C*. sp.2 aff. *sulawesiana* for the latter in their analysis. Morphologically, all the species of the *C. colocasiae-alocasiae* clade in the present study shared five stout spines on the foreleg second tarsomere and the conical process on the male sternite VI (Grimaldi, 1991; Sultana et al., 2006), and therefore the *C. colocasiae-alocasiae* clade corresponded to the *C. colocasiae* species subgroup (Takenaka et al., 2006).

In Sultana et al. (2006) the relationships among

Phylogenetic relationship of *Colocasiomyia* flies	Typical combination of species shareing the same host inflorescence	Breeding habit	Ovipositor	Host plant	Area of distribution	Reference
C. sp.X1			-	*Rhaphidophora hookeri*	Yunnan	Unpublished
C. gigantea		No breeding on the plant	-	*Colocasia gigantea*	Java	Okada, 1987; Takenaka, 2006
C. colocasiae		Stamenicolous	Nallow	*Colocasia esculenta*	The Oriental Region	Toda and Okada, 1987
C. sp.1 aff. *iskandari*		No data	Wide	*Colocasia esculenta*	Java	Takenaka, 2006
C. iskandari		No data	Wide	*Colocasia esculenta*	Java	Takenaka, 2006
C. steudnerae		Intermediate	Wide	*Steudnera colocasiifolia*	Yunnan	Takenaka et al., 2006
C. alocasiae		Stamenicolous	Nallow	*Alocasia odora*	Ryukyu~Yunnan	Okada, 1980
C. aff. *stamenicola*		Intermediate	Wide	*Alocasia odora*	Yunnan	Takano et al., Unpublished
C. xenalocasiae		Pistillicolous	Wide	*Alocasia odora*	Ryukyu~Yunnan	Okada, 1980
C. sp.1 aff. *diconica*		Pistillicolous	Wide	*Colocasia gigantea*	Java and Smatera	Takenaka, 2006
C. diconica		Pistillicolous	Wide	*Colocasia esculenta*	Myanmar~Indonesia	Toda and Okada, 1987
C. cristata		Pistillicolous	Wide	*Alocasia alba*	Java	Takenaka, 2006
C. sulawesiana		Pistillicolous	Wide	*Alocasia macrorrhizos*	Sulawesi	Okada and Yafuso, 1989; Takenaka, 2006
C. sp.1 aff. *sulawesiana*		Pistillicolous	Wide	*Alocasia macrorrhizos*	Borneo	Takenaka, 2006
C. sp.2 aff. *sulawesiana*		Pistillicolous	Wide	*Alocasia macrorrhizos*	Borneo	Takenaka, 2006

Figure 3 : Phylogenetic relationships among *Colocasiomyia* species and their breeding characteristics, host plants and geographical distributions.

the *C. colocasiae-alocasiae* clade, the *C. cristata-sulawesiana* clade and two independent species, *C. xenalocasiae* and *C. diconica*, were not resolved (Fig. 1). In the present analysis the two independent species formed a clade together with *C.* sp. aff. *stamenicola* and *C.* sp.1 aff. *diconica* and the relationships among the three monophyletic groups were resolved: the *C. cristata-sulawesiana* and the *C. xenalocasiae-diconica* clades are sister groups and the combined (*C. cristata-diconica*) clade is sister to the *C. colocasiae-alocasiae* clade (Fig. 2).

4.2 Evolution of the stamenicolous and pistillicolous breeding habits

The two sister groups, the *C. colocasiae-alocasiae* and the *C. cristata-diconica* clades, included 'typical' stamenicolous and pistillicolous species, respectively (Fig. 3). Okada (1986a) and Yafuso and Okada (1990) hypothesised the evolutionary origin and the dispersion of cohabitation between a stamenicolous and a pistillicolous species as follows: a couple of ancestral species of *Colocasiomyia* had established cohabitation on a certain plant of Araceae in a certain area of the Oriental Region, probably the southern part of Angara land. Tightly keeping the cohabiting relationship, they then evolved into *C. alocasiae* and *C. xenalocasiae* and dispersed in the direction of Taiwan and Okinawa on the one hand, and into *C. colocasiae* and *C. diconica* in Myanmar, Indochina to Java as well as into *C. stamenicola* and *C. pistilicola* (Carson & Okada, 1980) in New Guinea on the other.

The results of the present study seem to partially support their hypothesis for the relationship between the cohabiting pair of *C. alocasiae* and *C. xenalocasiae* and the pair of *C. colocasiae* and *C. diconica*: the divergence between stamenicolous and pistillicolous species may have occurred once at the base of the *C. cristata* group and the ancestral cohabiting pair radiated to several pairs on different host plants and at different locations: *C. alocasiae* and *C. xenalocasiae* on *Alocasia odora* in the area between Yunnan and Okinawa, *C. colocasiae* and *C. diconica* on *Colocasia esculenta* in Malesia and so on.

However, the *C. colocasiae-alocasiae* clade also includes *C. steudnerae*, *C. iskandari* and *C.* sp.1 aff. *iskandari*. *Colocasiomyia steudnerae* exhibits intermediate breeding habit between the stamenicolous and pistillicolous species (Takenaka et al., 2006) (Fig. 3). The breeding habits of *C. iskandari* and *C.* sp.1 aff. *iskandari* are unknown, but both species possess the wide ovipositors that are widely shared morphological characteristics among pistillicolous species, whereas stamenicolous species possess the narrow ovipositors that are presumably an adaptation for laying eggs in the narrower spaces between stamens. Taking these into account, the evolutionary pattern of the stamenicolous and pistillicolous breeding habits may have been more complicated than hypothesised by Okada (1986a) and Yafuso and Okada (1990).

4.3 Evolution of cohabitating pairs

Yafuso and Okada (1990) reported another pair of cohabiting species, *C. heterodonta* Yafuso & Okada, 1990 (stamenicolous species) and *C. xanthogaster* Yafuso & Okada, 1990 (pistillicolous species), from inflorescences of *Homalomena* spp. (Araceae). According to Sultana et al. (2002), the *C. toshiokai* group, which includes these two species, is regarded as an independent lineage of the *C. cristata* group (Fig. 1). Thus, cohabitation seems to have evolved repeatedly in the genus *Colocasiomyia*. In this sense, a cohabiting pair of *C.* sp.1 aff. *sulawesiana* and *C.* sp.2 aff. *sulawesiana* might give another example of parallelism of cohabitation (Figs 1 and 3). Although these two cohabiting species are generally pistillicolous in their breeding habits, they show a tendency of differentiation in reproductive nature and traits parallel to that between stamenicolous and pistillicolous species (Takenaka, 2006). This implies that they are in the initial phase of cohabitation with micro-allopatric niche differentiation. On the other hand, some pistillicolous *Colocasiomyia* species have been observed monopolizing their host plant inflorescences without cohabiting stamenicolous species: *C. cristata* on *Alocasia alba* (Araceae), *C. sulawesiana* on *Alocasia macrorrhizos* (but see Okada and Yafuso, 1989) and *C.* sp.1 aff. *diconica* on *Colocasia gigantea* (Fig. 3). The reason for the lack of cohabiting stamenicolous species is unclear yet, but it is notable that *C. cristata* and *C. sulawesiana* are intermediate in the female reproductive traits between *C.* sp.1 aff. *sulawesiana* and *C.* sp.2 aff. *sulawesiana* of the same clade (Takenaka, 2006).

4.4 Evolution of host selection

The members of the *C. xenalocasiae-diconica* and the *C. colocasiae-alocasiae* clades are widely distributed in the Oriental and Papuan regions and visit inflorescences of *Colocasia*, *Alocasia* and *Steudnera* species.

Colocasia esculenta is cultivated pan-tropically as an important crop plant today and is used as the host

plant by a number of *Colocasiomyia* species in the Oriental and Papuan regions. The region of its origin is still in dispute, but some researchers (Yen and Wheeler, 1968; Plucknett, 1976; Kurvilla and Singh, 1981; Matthews, 1995) believe that it is South-East Asia, probably within the Indo-Malayan region (but see Lebot, 1999). The host shifts between *Colocasia esculenta* and other plants such as of *Alocasia* and *Steudnera* may have occurred in the periphery of this region, e.g., southern China and the Sunda Islands.

For example, *C. alocasiae* and *C. xenalocasiae* might have sifted or expanded their host plants in the peripheral range of original host plant, probably *Colocasia esculenta*, because they breed on *Alocasia odora* in Taiwan and Ryukyu where inflorescences of *Colocasia esculenta* are not available, but utilize both plants in South China (M. J. Toda, pers. comm.).

The members of the *C. cristata-sulawesiana* clade exclusively utilise *Alocasia* species as host plants. At present the known distribution of this group is restricted to Borneo, Java and Sulawesi, which are known as the diversity centre of *Alocasia*. In addition to the species assigned to the *C. cristata-sulawesiana* clade in this study, *C.* sp.3 aff. *diconica* and *C.* sp.4 aff. *diconica*, which seem to be included in this clade (Toda et al., unpubl. data), have also been found monopolizing *Alocasia* inflorescences (Sultana et al., 2006). In the *C. cristata-sulawesiana* clade, the host shift from *Colocasia* to *Alocasia* might have occurred in couple with monopolization of host plant without cohabiting stamenicolous species (but see Okada and Yafuso, 1989).

5. Concluding remarks

This study showed that the evolutionary origin of the cohabitation, the diversification of the stamenicolous and pistillicolous breeding habits and the evolutionary route of the host selection in the *C. cristata* group are not so simple as previously thought by Okada (1986a) and Yafuso and Okada (1990). The plants so far recorded as hosts of the *C. cristata* group are still limited to about ten species of a few genera. Much more potential host plant species exist in the distribution range of the *C. cristata* group: for example, approximately ten *Colocasia* species are known from Indo-Malaya to the Sunda Islands (Mayo et al., 1997; Long and Liu, 2001; Cao and Long, 2003; Yin et al., 2004), 60–70 *Alocasia* species in the Oriental and Papuan regions to Northeastern Australia (Hay et al., 1997; Mayo et al., 1997; Hay, 1998; Yuzammi and Hay, 1998; Hay, 1999) and eight species of *Steudnera* in Indochina excluding Malay Peninsula (Mayo et al., 1997). Given the high host specificity of each species in the *C. cristata* group, a number of *Colocasiomyia* species might have been undiscovered. Biogeographic information and comparative ecological studies based on a reliable phylogenetic hypothesis with comprehensive taxon sampling of both *Colocasiomyia* flies and their host plants will give us deeper insights into the evolution and diversity of this highly mutualistic insect-plant interactions.

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逆位と遺伝子多型から追う アナナスショウジョウバエ亜群の種分化

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アナナスショウジョウバエ亜群における種分化を染色体変異と塩基配列に基づき検討した．*Drosophila bipectinata* complex 内の 4 種の関係は，染色体変異に基づく分岐図と分子レベルの分岐図が一致した．しかし，*D. ananassae* complex では染色体変異に基づく分岐図が分子レベルのものと一致しなかった．これは種分化後に種間交雑があり，遺伝子流動が生じたことを示唆する．古典的な染色体多型の分岐図をもとに塩基配列情報の解析を行うことにより，より詳細な系統関係が推定でき，遺伝子の種間移入の歴史が推測できる．

Evolutionary history of the *Drosophila ananassae* species subgroup inferred from chromosome arrangements and DNA sequences

Muneo Matsuda[1]

We compared chromosomal and molecular cladograms of three different taxonomic levels of the *Drosophila ananassae* species subgroup: 1) between complexes (the *ananassae, bipectinata* and *ercepeae* complexes, and *D. varians*), 2) between species within the *bipectinata* complex (*D. bipectinata, D. parabipectinata, D. malerkotliana*, and *D. pseudoananassae)*, and 3) between species within the *ananassae* cluster of the *ananassae* complex (*D. ananassae, D. parapallidosa, D. pallidosa*, *D. pallidosa*-like-WAU, and *D. papuensis*-like). Polytene chromosome data were consistent with molecular data within the *bipectinata* complex. On the other hand, we found that cladograms based on DNA sequence data were inconsistent with those from chromosomal arrangement data in the *ananassae* cluster. The inconsistency might be explained by gene flow caused by recent hybridization between species. Chromosomal arrangement (especially inversion) data would give us more information than nucleotide data alone.

1. 背景

Sturtevant and Dobzhansky（1936）および Dobzhansky and Sturtevant（1938）が核型や唾腺染色体に見られるバンドパターンの比較に基づきショウジョウバエの系統関係を研究する方法を発展させて以来，多くの研究成果が得られている（Dobzhansky, 1970; Carson, 1970; Lemeunier et al., 1986; Wasserman, 1992）．近年，DNA の塩基配列を用いた系統関係の構築が主流になり，多くの生物でその成果が報告されている．ショウジョウバエ 12 種のゲノム解析（Drosophila 12 Genomes Consortium, 2007）の進展とともに，アナナスショウジョウバエ亜群（*Drosophila ananassae* species subgroup）でも塩基配列をもとにした類縁関係が報告されている（Da Lage et al., 2007; Sawamura et al., 2010）．Da Large et al.（2007）は *Amyrel* 遺伝子の塩基配列の比較に基づき，*ananassae* 亜群は *ananassae* 種群とすべきとの提案をしているが，本稿では現在の分類に従い，*melanogaster* 種群に属する *ananassae* 亜群として議論を進める．*ananassae* 亜群は *ananassae*，*bipectinata*，*ercepeae* の 3 つの complex とこれらの complex には入らない *D. varians* を含め 24 種が記載され，熱帯～亜熱帯に汎世界的に分布している（Tobari, 1993; Lemeunier et al., 1997; Matsuda et al., 2009）．本論文では，現在までに得られている染色体から見た系統関係と分子レベルからみた系統関係を complex 間，*bipectinata* complex 内，*ananassae* complex の *ananassae* cluster 内の 3 分類階層について比較し，系統関係の相違点および類似点から新たな研究方向を提示したい．染色体から見た系統関係では分岐年代を追うことは困難であり，塩基配列から推定される情報には劣る．しかし，両者の解析を同時に行うことにより，逆位

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の切断点の構造解析，逆位と遺伝子との連鎖不平衡などの情報がより明確になることが期待される（O'Grady et al., 2001）．

2．結果

2.1 *ananassae* 亜群に属する complex 間の分化

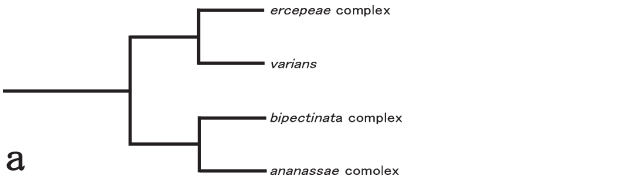
Da Lage et al.（2007）の *Amyrel* 遺伝子の変異のデータに基づき作成した種の分岐図（cladogram）を図1aに示した．この分岐図は *CO1*，*Ddc*，*Gpdh*，*Pgi* 遺伝子に基づく系統樹（Matsuda et al., 2009）と矛盾しない．*ananassae* complex＋*bipectinata* complex と *ercepeae* complex＋*D. varians* の2つに分けられていることが特徴的である．一方，染色体情報を用いた分岐図はこれとは異なる．第1精母細胞における X，Y，第4染色体の対合によって形成される4価染色体の有無（Matsuda et al., 1983; Matsuda et al., 2009）と rDNA の染色体上の位置（Roy et al., 2005; Shibusawa et al., 2007）から，*ercepeae* complex とその他の種（*D. varians*，*bipectinata* complex，*ananasae* complex）に分けられる（図1b）．X 染色体と第4染色体の間で起こったとされる *D. ananassae* の転座（Kikkawa, 1938）は *ercepeae* complex と分岐した後であることを示唆している（図1b）．また，*bipectinata* complex の4種すべてにおいて自然集団中にB染色体が観察される（Matsuda et al., 2005）．これは本 complex の特徴であり，現在まで *ananassae* 亜群の他の種では報告されていない．この結果は塩基配列から得られた分岐図と矛盾しない．

2.2 *bipectinata* complex 内での種分化

Kopp and Barmina（2005）が解析した6つの核遺伝子（*al*，*bab2*，*Gpdh*，*ple*，*Sod*，*up*）の塩基配列に基づく分岐図を図2aに示した．*D. malerkotliana*，*D. parabipectinata*，*D. bipectinata* が単系統群を形成し，*D. pseudoananassae* が最も古く分岐したことを示している．また，*D. malerkotliana* と *D. parabipectinata* との塩基配列の相違は *D. malerkotlina* と *D. bipectinata* 間および *D. parabipectinata* と *D. bipectinata* 間よりも大きく，種間の生殖的隔離の関係とほぼ同じである（Kopp and Barmina, 2005）．Tomimura et al.（2005）は，*bipectinata* complex 全種に共通な標準唾腺染色体地図を作成し，4種間の系統関係を明らかにした．その結果に基づく4種の分岐図を図2bに示した．この結果は塩基配列から作成された分岐図とは矛盾せず，*D. pseudoananasase* とそれ以外の3種が単系統群を形成している．*D. malerkotliana*，*D. parabipectinata*，*D. bipectinata* 間の塩基配列変異量と染色体逆位数とは必ずしも相関しない．これらの相違は両論文（Kopp and Barmina, 2005; Tomimura et al., 2005）でも指摘されているように，雑種形成の可能性と集団の大きさの違いを反映していると考えられる．本 complex 内で最も分布が広いのは *D. malerkotliana* であり，熱帯～亜熱帯で汎世界的に観察される．次いで分布が広いのは *D. bipectinata* であり，広くアジア，オセアニア，南太平洋諸島に分布している（Matsuda et al., 2005）．他の2種はアジア，パプアニューギニアの限られた地域に分布し，その集団の有効サイズは大きくないと推測される．

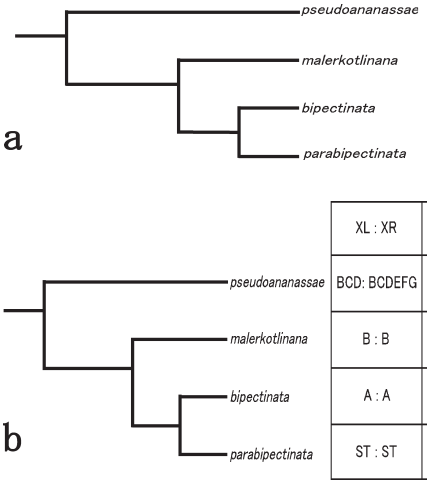
2.3 *ananassae* cluster 内での種分化

ananassae complex 内で交配可能な種（*ananassae* cluster）において核遺伝子（*Actn*，*w*，*CG7785*，



	locations of rDNA			male meiosis	B-chromosome
	X	Y	4th	X-Y-4h teravalent	
ercepeae complex	+	+	−	No	−
varians	−	−	+	Yes	−
bipectinata complex	−	−	+	Yes	+
ananassae complex	−	+/−	+	Yes	−

図1：(a) *Amyrel* 遺伝子の塩基配列に基づく *ananassae* 亜群の分岐図〔Da Lage et al.（2007）を参考に作成〕．(b) *rRNA* 遺伝子の染色体上の位置，雄減数分裂における X，Y，第4染色体の対合像，およびB染色体の有無から見た *ananassae* 亜群の分岐図〔Roy et al.（2005），Shibusawa et al.（2007），Matsuda et al.（2005，2009）を参考に作成〕．



XL : XR	2L : 2R	3L : 3R
BCD: BCDEFG	? : ABKLM	AFG : ALP
B : B	A+CQ : AB/ABK	A : AL
A : A	A+B : AB/A	A+CD/A : ABC
ST : ST	ST : ST/AB	ST : ST/A

図2：(a)核遺伝子（*al*, *bab2*, *Gpdh*, *ple*, *Sod*, *up*）の塩基配列に基づく *bipectinata* complex の分岐図〔Kopp and Barmina（2005）を参考に作成〕．(b)逆位の種間変異に基づく *bipectinata* complex の分岐図〔Tomimura et al.（2005）を参考に作成〕；各染色体腕のアルファベッドは染色体の逆位を表している．

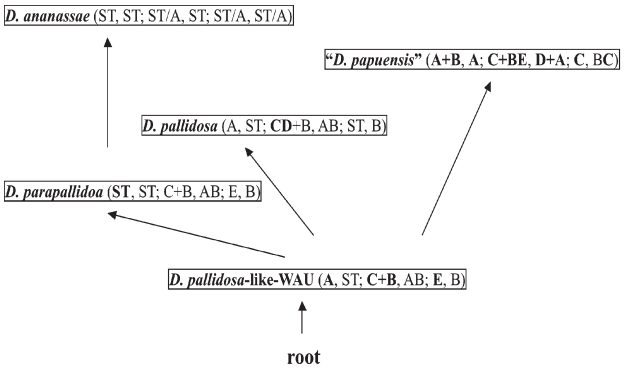


図 3：唾腺染色体の配列（XL. XR; 2L, 2R; 3L, 3R）に基づく *ananassae* cluster の系統関係〔Matsuda et al.（2009）を参考に作成〕.

ZnT63C）のイントロン領域の塩基配列に基づく分岐図の作成を試みたが，遺伝子によって分岐図が異なり，4 遺伝子座をまとめた図は描けていない（Sawamura et al., 2010）．同様に，ミトコンドリアの *CO1* 遺伝子を用いたハプロタイプのネットワーク（Matsuda et al., 2009）においても明確な分岐図は描けず，近年に交雑が起こった可能性を示唆する．主に唾腺染色体の配列に基づいて，*ananassae* cluster には *D. ananassae*，*D. pallidosa*，*D. parapallidosa*，*D. pallidosa*-like，*D. papuensis*-like，*D. pallidosa*-like-WAU が報告されている（Tomimura et al., 1993; Matsuda et al., 2009）．しかし，*D. pallidosa*-like は系統によって他種との間の生殖的隔離の程度が異なり，種としてのまとまりは疑わしい（Matsuda et al., 2009）．また，Futch（1966）は南太平洋地域の *ananassae* の中に体色が明るい *D. pallidosa* を区分し，さらにパプアニューギニアでは特異的な逆位を持つ系統を発見して，〝papuensis〟と仮称した．我々は 1981 年にパプアニューギニアおよび南太平洋諸島で採集された系統を解析し，パプアニューギニアの集団に〝papuensis〟とほぼ同じ逆位を持つ系統を発見した（Tomimura et al., 1993）．しかし，この系統には 1966 年には報告されていなかった新たな逆位とともに，*D. ananassae* のほとんどの集団で観察される遺伝子配列の染色体（ST）があり，近年に *D. ananassae* との雑種が形成された可能性を示した（Matsuda et al., 2009）．我々はこれを *D. papuensis*-like とよんでいる．しかし，強い生殖的隔離が他種との間で認められ，Futch（1966）の結果を再現することができた．そこで，遺伝子移入前の染色体配列を推測し，系統関係を示したのが図 3 である．この結果は Y 染色体連鎖の遺伝子（*k12*）の塩基配列に基づき作成した系統樹（Matsuda et al., 2009）と矛盾しない．

3．考察

D. ananassae は 2007 年にゲノム解析が発表された 11 種のショウジョウバエの 1 種（*Drosophila* 12 Genomes Consortium, 2007）であり，その後の種分化や比較ゲノム研究の一翼を担う種となってきた．さらに，同亜群の *bipectinata* complex に属する *D. bipectinata* ゲノムの塩基配列解析もほぼ終了し（A. Kopp，私信），*ananassae* 亜群内および *melanogaster* 種群の種分化機構を研究するモデル生物となった．しかし，亜群内の complex 間や complex 内（特に cluster 内）においては，ミトコンドリアや核の遺伝子レベルだけで系統関係を構築することは限界がある（遺伝子によって異なった系統樹が描かれる）ことがわかってきた．特に交配可能な種間の比較では，雑種形成による遺伝子移入を無視できず，移入された遺伝子が遺伝的な組み換えによって集団内に広まる可能性がある．この問題を解決し，信頼性の高い系統関係を示すには，組み換えがほとんど無視できる Y 染色体上の遺伝子や唾腺染色体から推測した種特異的逆位の切断点近傍の遺伝子の塩基配列変異を調べる方法が考えられる．幸いに *D. ananassae* では唾腺染色体のバンドと塩基配列情報との関係が明らかになっている（Shaeffer et al., 2008）ので，切断点近傍の遺伝子座を選ぶことが可能である．このような試みはすでに *D. pseudoobscura* とその近縁種で行われており（Noor et al., 2007），*ananassae* cluster でも Sawamura et al.（2010）にその一端が報告されているが，十分な解析は行われていない．今後，逆位と連鎖した複数の遺伝子座の解析をさらに進める必要がある．

塩基配列と染色体変異から推測した分岐図に矛盾が認められなかった *bipectinata* complex 内の種は，すでに長期にわたり種間雑種の形成が野外ではほとんど起きていないことを示唆し，塩基配列をもとに比較的正確な類縁関係を構築することができる分岐年代の種であると推定される．Kopp and Barmina（2005）によれば，*D. bipectinata*，*D. parabipectinata*，*D. malerkotliana* の分化は 283,000〜385,000 年前と推測している．このことは，唾腺染色体の逆位と塩基配列情報の結果を結びつけることができて初めて明らかになった点である．また，本 complex で同定された種内，種間の逆位の切断点の分布を見ると，唾腺染色体のバンドレベルではあるが，切断点にホットスポットがあることが示唆されている（Tomimura et al., 2005）．また，*D. ananassae* complex と同様に第 2 染色体左腕（マラーのエレメント E）に最も多くの逆位が報告されている．*ananassae* 亜群内で見られる共通の現象は，種分化に特定の染色体や逆位が重要な働きをしている可能性を示唆する．他の種群との比較とともに染色体腕の特徴も今後の研究対象である．

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Chromosomal variation of the leaf-mining *Liodrosophila castanea* (Diptera, Drosophilidae), with the results of crossing experiments

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Chromosomal variation was found in three insular populations of *Liodrosophila castanea* (Diptera, Drosophilidae). Both Yakushima and Okinawa strains possessed 2n=12 chromosomes, 5 pairs of acrocentric autosomes and sex chromosomes, in the metaphase plates of larval brain cells. Of them a pair of middle-sized autosomes were heteropyknotic from the prophase to early metaphase during the cell division. Tsushima strain had 2n=10 chromosomes, 4 pairs of acrocentric autosomes and sex chromosomes. All nuclear plates of Tsushima strain contained small supernumerary chromosomes ranging from 2 to 9 in number and lacked any heterochromatic autosome. Morphologically, any discriminative character was not found between different chromosomal strains, excepting the nearly bare cercus in Yakushima and Okinawa strains (pubescent in Tsushima's). Male flies mated well with different strain females in crossing experiments without any mating preference, and produced fertile F_1 males and females in both directions of crosses. The present results demonstrate that these insular strains with different karyotypes should be regarded as chromosomal races of *Liodrosophila castanea*.

1. Introduction

Liodrosophila castanea was described by Okada and Chung (1960) from Chon-Nam district, Huk-san Island, Korea, and since then its collection records had been restricted to southern parts of the Korean Peninsula including adjacent islands (Lee, 1966; Okada, 1988). We had a chance to collect drosophilid flies in three islands, Tsushima, Yakushima and Okinawa, of southern Japan, and obtained questionable specimens by net sweeping on herbaceous plants. Prof. M. J. Toda (Hokkaido University) kindly identified the specimens as *L. castanea*, and noticed slight morphological variations in the specimens from Yakushima and Okinawa.

We carried out karyotype analyses in a total of 11 iso-female strains from these local populations and found chromosomal variation. Then, we conducted crossing experiments for examining the reproductive isolation and compared the morphology, including the genitalia, between the different chromosomal strains.

2. Material and methods

2.1 Geographic strains

Eleven strains of *L. castanea* were employed: four were collected at Sumo, Tsushima Island (henceforth designated as TS), Nagasaki Prefecture, in September, 1996, four at Kurio, Yakushima Island (YK), Kagoshima Prefecture, in October, 1996, and three at Experimental Forests of Ryukyu University, Yona, Okinawa Prefecture (OK), in March, 1997 and in September, 1998. All living strains used for karyotype observation and crossing experiments were established from a single inseminated female caught in the field.

2.2 Rearing method

Liodrosophila castanea is a leaf-mining species and cannot be maintained in standard *Drosophila* medium. A new culturing method was adopted in the present study. Leaves of *Spinacia oleracea* L. were mashed by a blender machine and then frozen in refrigerator to kill larvae of other insects. They were placed on

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filter paper, added with a small piece of apple for inhibiting mold, and then kept in a glass vial (30 mm in diameter, 100 mm in height). For establishing an iso-female strain, a wild-caught female was slightly etherized and identified, and kept in a glass vial with food described above at 18°C under continuous light. Mashed leaves were added on the shortage of food for larvae. Hatched larvae entered into a layer of mashed leaves and filter paper with sap, developed well in the layer and pupated on its surface.

2.3 Chromosome preparation

Neuroblasts of 3rd instar larvae were used to prepare mitotic chromosomes as follows: treated with a mixture solution of 0.1mg/ml of colchicines and 1% of sodium citrate for 45–60 min, fixed with a solution (carbinol: acetic acid=3: 1) for 30 min, stained with 4% Giemsa solution for 60 min, and then air-dried (Imai et al., 1977). About 30 nuclear plates were examined for each strain. Photos of metaphase chromosomes were taken by an analog camera (Olympus PM-6) and then saved as digital figures using a film-scanner (Nikon APS IX240).

2.4 Morphological observation

We compared some metric characters and the genital structures between the strains with different chromosome constitutions in the present study. Male and female terminalia were detached from the body, and treated with 10% KOH solution at 80°C for several minutes, and observed in a droplet of glycerin under a compound microscope. Twenty-three quantitative characters (Table 1) were measured with an ocular micrometer: body length (BL), thorax length (TL) including scutellum, wing length (WL), wing width (WW), ratio of frons/head width (FW/HW), numbers of dorsal (db of Ar) and ventral branches (vb of Ar) of arista, gena/maximum width of eye (G/E), length ratio of 2nd oral/vibrissa (Or2/Or1), length ratio of proclinate/posterior reclinate seta (Orb3/Orb1), length ratio of upper/lower humeral setae (U/L of Hu), length ratio of anterior/posterior dorsocentral setae (DcA/DcP), length distance/cross distance of dorsocentals (Ld/Cd of Dc), length ratio of posterior/anterior scutellar setae (SctP/SctA), distance from SctA to Sctp/distance between SctPs (SctA-SctP/SctPs), sterno-index, and seven wing indices (costal, 4V, 4C, 5x, Ac, M, C3-fringe). The first four characters, BL, TL, WL and WW, were measured separately for each sex.

Some quantitative characters such as the body size are quite variable according to rearing conditions. Five of mature females were allowed to oviposit in a glass vial with food, and the vials were renewed every three or four days, to avoid the overpopulation of larvae. When young flies emerged, they were transferred to a new vial at the same intervals. Twenty individuals (10 males and 10 females) were selected randomly and examined.

2.5 Crossing experiments

When adult flies emerged, they were collected every 24 hrs, sorted for sexes, and maintained in vials. All crossing experiments were carried out using 8- to 12-day-old flies, since *L. castanea* is a relatively rapid breeder and most adults reach to the mature stage for reproduction within 8 days after eclosion. Five males and five alien or same-strain females were put together into a vial (30 mm in diameter, 100 mm in height). After 48 hrs, the females were taken out, dissected in Ringer's solution, and examined for sperm in the spermathecae and the seminal receptacle. About 50–100 females were checked in each crossing. The index of pre-zygotic isolation for each cross-pair was estimated by the formula of Coyne and Orr (1989, 1997): 1– (% of heterogamic matings (i.e., A♀×B♂)) / (% of homogamic matings (A♀×A♂)). This index gives the value of 1 when isolation is perfect and the value of 0 for free crossings.

For mate-choice experiments, the choice-by-male method was adopted. The tip of the right or left wing was marked with red ink for recognizing the strains. Five males and ten (five own and five alien-strain) females were placed together, and the females were dissected in Ringer's solution for sperm after 48 hrs. The degree of mating preference was evaluated by Stalker's (1942) isolation index (I.I.): I.I.=[% of Homogamic (+) − % of Heterogamic (+)]/[% of Homogamic (+) + % of Heterogamic (+)], where Homogamic (+) and Heterogamic (+) mean females inseminated by same-strain and alien-strain males, respectively. This formula gives the value of 1 for completely assortative mating preference and 0 for lack of mating preference.

When hybrid adults were obtained, F_1 fertility was checked by backcrossing between the hybrids and the parental strains.

3. Results and discussion

Figs 1A and B show the male metaphase configu-

rations of the Yakusima (YK) and Tsushima (TS) strains of *L. castanea*, respectively. This is the first report on the karyotype of the genus *Liodrosophila* (Clayton and Guest, 1986; Clayton, 1998). The metaphase configurations of YK comprised 12 chromosomes in the diploid number with 5 pairs of acrocentric autosomes, relatively large acrocenric X, and submeta- or meta-centric Y. Two autosomes indicated with the arrows in Fig. 1A were denser in condensation than the other autosomes, probably due to heteropyknosis, from the prophase to early metaphase during the cell division, which implies rich heterochromatic elements of these autosomes. The same chromosomal constitution of 2n=12 with a pair of heteropyknotic autosomes was found in three Okinawa (OK) strains of *L. castanea*.

On the other hand, brain cell preparations of TS strains showed 2n=10 chromosomes, and all were acrocentric. Metaphase plates of TS lacked two heterochromatic autosomes found in those of YK and OK and had small supernumerary chromosomes (indicated with the arrows in Fig. 1B). These dot-like chromosomes were densely stained, and its number per nuclear plate was quite variable, ranging from 2 to 9, so far as we examined about 240 cells.

Figs 1C and D show the metaphase plates of hybrids in the crosses between YK females and TS males and in its reciprocal crosses, respectively. All nuclear plates of F_1 larval brain cells showed 2n=11 chromosomes and a few of dot-like supernumerary ones (indicated with the arrows in Figs 1C, D), suggesting that in the nuclear plate of F_1 offspring six chromosomes might have derived from YK but the other five and a few dot-like chromosomes from TS.

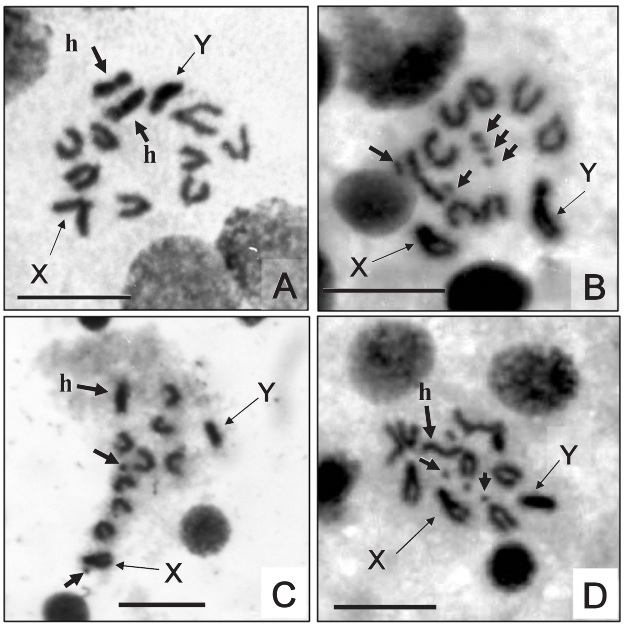
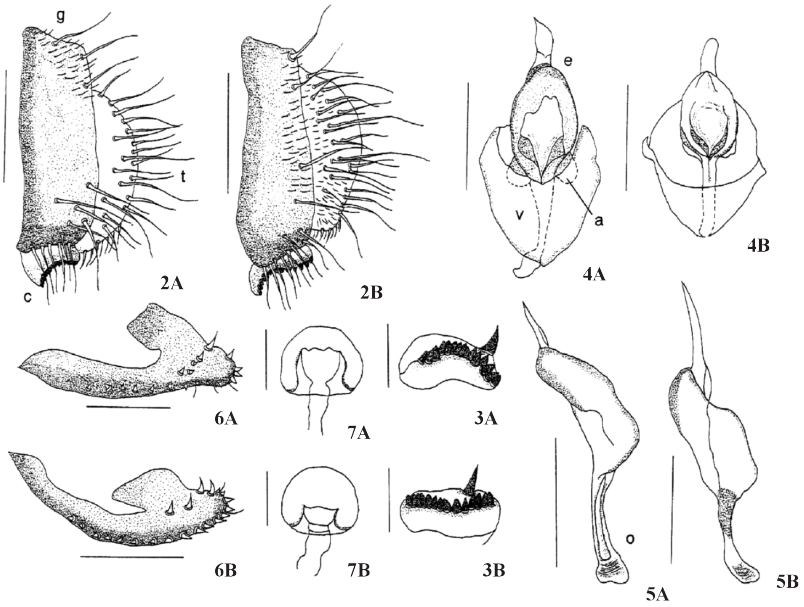


Figure 1：Male metaphase chromosomes of *Liodrosophila castanea*. A, Yakushima strain; B, Tsushima strain; C, F_1 in the cross between Yakushima♀×Tsushima♂; D, F_1 in the cross between Tsushima♀×Yakushima♂. Bars=10μm.

Figs 2–7 show the male and female genitalia of YK (A) and TS (B) strains, respectively. The genital characters of YK were as follows: epandrium dark brown, pubescent on dorsal portion, with ca. 3 setae on upper, ca. 5 setae on lower and ca. 6 setae along ventral margin (Fig. 2A); surstylus with ca. 15 stout setae and a large seta near the apex on inner surface (Fig. 3A); cercus almost bare, hemispherical, with ca. 18 long setae and several short setulae on ventral margin (Fig. 2A); aedeagus grayish brown, apically pointed, club-shaped at lateral view (Figs 4–5A); aedeagal apodeme grayish brown (Fig. 5A); hypanrium narrowing distally (Fig. 4A); paramere brown, minute; oviscapt black on ventral margin, roundish at caudal tip, with ca. 17 orange marginal and ca. 5 lateral ovisensilla (Fig. 6A); spermatheca brown, with introversion half of outer capsule height (Fig. 7A). Of them, the most remarkable difference was seen in the pubescence on the cercus between YK and TS: the cercus was bare in YK, but widely pubescent in TS (Fig. 2B). In addition, the epandrium was pubescent only on the upper portion in YK, but on medial to upper portion in TS *castanea* (Figs 2A, B), and the spermathecal introversion duct was slightly higher in YK than in TS (Figs 7A, B). However, these characters in the epandrium and spermatheca varied among individuals within the same local strains, with the extreme case of YK strains resembling TS strains. Any discriminative difference was not detected in the surstylus (Fig. 3B), the aedeagus and its adjacent organs (Figs 4–5B), or the female terminalia (Fig. 6B) between YK and TS strains.

Table 1 gives the results of comparisons for the 23 quantitative characters between YK and TS strains. Twelve out of 23 characters (Nos. 2–4, 9, 10, 14, 15, 18–22) showed statistically significant differences between the strains, but with overlapped ranges between them in all characters.

Table 2 gives the percentage ratios of successful mating between YK and TS strains in no-choice method. The two strains mated well with each other in both directions, but the ratios of successful mating were slightly lower in the crosses between the differ-



Figures 2–7：Male and female genitalia of Yakushima (A) and Tsushima (B) strains of *Liodrosophila castanea*. 2, Epandrium and cercus; 3, surstylus; 4, aedeagus and adjacent organs in ventral view; 5, aedeagus in lateral view; 6, oviscapt; 7, spermatheca. Abbreviations: a, paramere; c, surstylus; e, aedeagus; g, epandrium; o, aedeagal apodeme; t, cercus, v, hypandrium. Bars: 0.1 mm in Figs. 1, 5; 0.05 mm in other figures.

Table 1：Variations of quantitative characters in two chromosomal strains of *Liodrosophila castanea*

Quantitative character*		Yakushima			Tsushima			Difference between strains (t-test)
		Mean±S.D.	Range	(n)	Mean±S.D.	Range	(n)	
1. BL(mm)	♂	1.96±0.14	1.80–2.20	(10)	1.86±0.23	1.60–2.36	(10)	ns
	♀	2.12±0.16	1.81–2.40	(10)	2.12±0.27	1.76–2.68	(10)	ns
2. TL(mm)	♂	0.77±0.05	0.72–0.88	(10)	0.70±0.04	0.66–0.80	(10)	$P<0.01$
	♀	0.81±0.05	0.72–0.88	(10)	0.76±0.05	0.68–0.84	(10)	$0.01<P<0.05$
3. WL(mm)	♂	2.02±0.10	1.84–2.12	(10)	1.79±0.08	1.68–1.92	(10)	$P<0.01$
	♀	2.08±0.07	1.96–2.20	(10)	1.94±0.08	1.84–2.12	(10)	$P<0.01$
4. WW(mm)	♂	0.77±0.04	0.72–0.84	(10)	0.66±0.02	0.62–0.72	(10)	$P<0.01$
	♀	0.80±0.03	0.76–0.84	(10)	0.73±0.06	0.68–0.88	(10)	$P<0.01$
Head								
5. FW/HW		0.50±0.02	0.47–0.53	(20)	0.49±0.02	0.45–0.56	(20)	ns
6. db of Ar		4.75±0.44	4.0–6.0	(20)	4.45±0.60	4.0–6.0	(20)	ns
7. vb of Ar		2.00±0.00	2	(20)	2.00±0.00	2	(20)	ns
8. G/E		0.21±0.05	0.13–0.30	(20)	0.20±0.03	0.15–0.27	(20)	ns**
9. Or2/Or1		0.78±0.11	0.60–1.00	(20)	0.90±0.11	0.67–1.00	(20)	$P<0.01$
10. Orb3/Orb1		0.62±0.05	0.50–0.69	(20)	0.68±0.05	0.60–0.75	(20)	$P<0.01$
Thorax								
11. U/L of Hu		0.96±0.10	0.67–1.00	(20)	1.01±0.08	0.86–1.33	(20)	ns
12. DcA/DcP		0.71±0.07	0.62–0.85	(20)	0.65±0.08	0.44–0.79	(20)	ns
13. Ld/Cd of Dc		0.62±0.05	0.54–0.74	(20)	0.55±0.08	0.48–0.77	(20)	ns**
14. SctP/SctA		0.65±0.11	0.33–0.79	(20)	0.56±0.06	0.45–0.65	(20)	$P<0.01$**
15. SctA-SctP/SctPs		0.72±0.08	0.57–0.86	(20)	0.64±0.05	0.53–0.71	(20)	$P<0.01$
16. Sterno-index		0.30±0.08	0.18–0.50	(20)	0.26±0.05	0.20–0.36	(20)	ns**
Wing indices								
17. C		1.61±0.11	1.46–1.87	(20)	1.63±0.12	1.45–1.90	(20)	ns
18. 4V		1.83±0.08	1.68–1.94	(20)	1.91±0.10	1.65–2.06	(20)	$P<0.01$
19. 4C		1.30±0.05	1.21–1.41	(20)	1.36±0.09	1.21–1.57	(20)	$0.01<P<0.05$**
20. 5x		1.65±0.14	1.42–1.90	(20)	1.61±0.18	1.38–2.00	(20)	$P<0.01$
21. Ac		3.48±0.26	3.07–4.17	(20)	3.64±0.32	3.08–4.40	(20)	$P<0.01$
22. M		0.52±0.04	0.45–0.59	(20)	0.49±0.05	0.42–0.58	(20)	$0.01<P<0.05$
23. C3-fringe		0.60±0.06	0.50–0.71	(20)	0.63±0.06	0.55–0.80	(20)	ns

* Abbreviations are explained in the text.
** Aspin-Welch method was adopted in the case of unequal variance (F-test, $\alpha=0.05$).

ent strains than in the crosses within the same strain. Although asymmetric mating preference has been frequently reported in closely related species or subspecies of *Drosophila* (Stalker, 1942; Watanabe and Kawanishi, 1979; Coyne and Orr, 1997; Wang et al., 2007), the indices of pre-zygotic isolation were nearly identical between the reciprocal crosses, being 0.338 between YK females and TS males and 0.275 between TS females and YK males.

Table 3 shows the results of crosses by the choice-by-male method. In both crosses the isolation index was nearly 0, indicating the absence of mating preference of males for females with different karyotypes.

We maintained the culturing vials containing both YK and TS flies for 2 weeks, and hybrid offspring appeared in both crossing directions. In the backcross, F_1 males mated to its parental females and produced fertile adults. Further, any syndrome of hybrid deficiency, such as viability, longevity or fecundity, was not observed in the adult flies obtained in the backcrosses or of F_2. This means a lack of post-mating isolation between the two chromosomal strains.

It is reasonable to assume that extra-chromosomes involved in the nuclear plates of TS strains might have derived from fragmentations of heterochromatic autosomes in those of YK and OK strains, and thus the latter would be more primitive in the karyotype evolution. At the present knowledge, these two types of *L. castanea* are allopatric in the geographic range, but any reproductive isolation was not observed in the crosses between these strains. These results imply that two geographic populations with the different karyotypes can be regarded as chromosomal races, rather than subspecies, although a slight morphological difference was found in the male genitalia (Mayr, 1974; Stamos, 2003).

Supernumerary chromosomes have been known in many taxonomic groups of invertebrates: for example, White (1972) demonstrated the chromosomal evolution and speciation by extra chromosomes in the grasshopper *Moraba viatica*. However, adaptive significance of extra-chromosomes in the natural population of *L. castanea* is quite open to question. This species has been presently known from Korea, China and southern districts of Japan (Okada 1988; Zhang et al., 1996; Bächli, 2007), and further studies on the karyotype and biology of this species are indispensable to clarify its genetic differentiation in relation to adaptation to local environments in many populations, especially in Kyushu locating between Tsushima and Yakushima.

Table 2 : Percentages of inseminated females in the crosses between two geographic strains of *Liodrosophila castanea*, Yakushima (YK) and Tsushima (TS), by no choice method. The numbers in parentheses and square brackets give the number of females examined and the pre-zygotic isolation index by Coyne and Orr (1989), respectively.

♂/♀	YK	TS
YK	82.4(68)	59.7 (67)[0.275]
TS	49.3(71)[0.338]	74.5(145)

Table 3 : Numbers and percentages (in parentheses) of females inseminated (+) and uninseminated (−) in crosses between two geographic strains of *Liodrosophila castanea*, Yakushima (YK) and Tsushima (TS), by choice-by-male method, and the calculated sexual isolation indices (I.I.) with the results of statistical tests.

Crosses		Homogamic		Heterogamic		I.I.
Female	Male	+	−	+	−	
YK+TS	YK	38(82.6)	7(17.4)	27(56.3)	21(43.7)	0.189^{ns*}
YK+TS	TS	34(72.3)	13(27.7)	28(59.6)	19(40.4)	0.096^{ns}

* ns: statistically non-significant.

Acknowledgements

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キイロショウジョウバエ種群における交尾器形態の進化：その機能研究に関するレビュー

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動物の交尾器，特にオス交尾器の形態は，他の形態形質よりも進化・多様化が速い傾向にあり，その機構について議論が続いている．本稿では，モデル生物であるキイロショウジョウバエを含むキイロショウジョウバエ種群について，交尾器形態の機能とその進化に関わる研究についてレビューした．オス交尾器による創傷行動の発見により，従来の研究では見出されてこなかった，オス交尾器に対応した多様性がメス交尾器の側にも同定されるようになった．しかし，メス交尾器の多様性は，柔軟な膜質構造の形態変化によるものが多い．交尾時の雌雄交尾器の対応関係の把握は，そのような旧来の手法では観察の難しい構造の特定を容易にし，種間比較や操作実験による機能の研究に足がかりを与える．そのため手法の一つとして，交尾中ペアを透明化する技術を紹介し，本種群が交尾器研究のモデル系となる可能性を示す．

The evolution of genitalia in the *Drosophila melanogaster* species group: a review on the studies of genital functions

Yoshitaka Kamimura[1], Hiroyuki Mitsumoto[1]

Animal genitalia, especially those of males, generally evolve more rapidly compared to other morphological traits. The driving forces underlying such evolutionary trends are much debated. In the present article, we review evolutionary and functional studies on the genitalia of drosophilid flies (Diptera) belonging to the *Drosophila melanogaster* species group. Despite that the male genitalia of this group show astonishing diversity, previous studies failed to detect any species-specific differences in female morphology that corresponded to interspecific differences in the male genital morphology. However, recent discovery of copulatory wounding behavior, that is, the phenomenon that male genitalia inflict wounds on the genitalia of the mate, enabled us to identify counter-evolutions in the female genitalia. Such diversification in the female genitalia is mainly represented as modifications in the soft membraneous regions. With description of a newly developed transparentizing technique of mating pairs, we discuss the potential of this species group to be a model research system of genital evolution.

1. はじめに

体内受精をおこなう動物の多くでは，オスは交尾器を用いてメスに精子を渡し，メスはそれを受け取り，卵の受精に必要な時まで貯蔵する．精子の受け渡しがオスの交尾器の基本機能であるが，交尾器形態はこのような単純な基本機能のみでは説明できないくらい多様であることが多い．昆虫類を含む多くの動物において，分類学の専門家が交尾器を観察しなければ，その動物が一体何者であるのかを正しく同定できない場合も多く，このことは，動物の他の外部形態よりも，交尾器は速く進化していく傾向があることを示している（Eberhard, 1985; Hosken and Stockley, 2004）．その急速な進化をもたらす要因は何であろうか？　最も古くから知られる仮説は，雌雄が正しい同種の交尾相手を他種から識別するために，種特異的な交尾器形態が進化したとする考えである（Shapiro and Porter, 1989）．この鍵と鍵穴（lock and key）仮説と呼ばれる考えによれば，交尾開始後に交尾器の形態によって同種か否かが認識でき，もしも別種であれば交尾を中断することで卵（または精子）の浪費を回避することができると期待される．しかし，近縁種と遭遇する機会の少ない寄主特異性の高い動物（例えばシラミ類）でも種特異的な交尾器形態の進化が観察されるなど，この仮説を強く支持する実例は乏しい

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（Eberhard, 1985; Hosken and Stockley, 2004）．現在では，精子競争（メスの体内で複数のオスに由来する精子が卵の受精をめぐる競争関係に置かれること），あるいはメスによっておこなわれる精子の選択，交尾の開始や継続時間をめぐる雌雄の対立といった，交尾開始以後に作用する性選択の働きを重視する考え方が一般的となりつつある（Eberhard, 1985; Hosken and Stockley, 2004; Simmons et al., 2009）．*Drosophila melanogaster*（キイロショウジョウバエ）は，遺伝学・分子生物学・発生生物学上の重要なモデル生物であるが，交尾器形態の進化研究においては，モデル生物として研究を先導しているとは言えないのが現状である．本稿では，ショウジョウバエ属（*Drosophila*），シマショウジョウバエ亜属（*Sophophora*）に属するキイロショウジョウバエ種群（*D. melanogaster* とその近縁種を含む）を対象に，本グループの交尾器形態の進化研究に関する現状，課題，展望についてまとめてみたい．

キイロショウジョウバエ種群（the *Drosophila melanogaster* species group）は 200 種近くが記載されている大きなグループで，12 の亜群（種亜群 species subgroup）に分けられる（表 1；Bock, 1980; Lemeunier et al., 1986; Toda, 1991）．ショウジョウバエ科（Drosophilidae）では 12 種についてそのゲノム情報の解読が完了しているが（Drosophila 12 Genomes Consortium, 2007），そのうち半数の 6 種が本種群に属している．種分化研究，さらに近年では進化発生学上のモデルとしても広く利用されており（例えば Prud'homme et al., 2006），分子生物学的・遺伝学的情報が最も蓄積している生物群の一つと言える．

本種群に特徴的な形質としては，腹部背板に分断されない黒帯があること，オスは前肢跗節に性櫛（sex comb）を持ち，腹端が黒化すること，メスの管状受精嚢（ventral receptacle；交尾の際にオスから受け取った精子を貯蔵する器官の一つ）やオスの精巣は長く，後者はコイル状になることなどがあげられるが，各特長を個別にみると例外も多い（Sturtevant, 1942; Bock and Wheeler, 1972; Bock, 1980; Lemeunier et al., 1986）．しかし，材料の入手や飼育が困難な一部の亜群（*D. longissima* 亜群，*D. denticulata* 亜群）は解析に含まれていないものの，これまでの分子系統解析の研究結果は本種群が単系統であること支持している（例えば Kopp, 2006; Da Lage et al., 2007; van der Linde et al., 2010）．研究結果は多くの亜群（*D. montium*, *D. ananassae*, *D. melanogaster* 各亜群など）も単系統であることを支持しているが，*D. suzukii* 亜群については多系統と考えられている（Schawaroch, 2002; Kopp, 2006; van der Linde et al., 2010）．*D. montium* および *D. ananassae* 両亜群についてはそれぞれを種群として独立させる分類体系も提案されているが（Da Lage et al., 2007），ここではこれらもキイロショウジョウバエ種群に含めて扱うことにする．

ショウジョウバエ類の交尾器形態の進化の話題として，しばしばメスの体内にある管状受精嚢の長さと，精子の長さ（あるいは精巣長）が比例する例が取り上げられる．この話題に関しては多くの優れた研究があるが（例えば Joly and Schiffer, 2010），ここでは交尾器を「交尾の際に雌雄の直接的接触がある部位」と狭義にとり，これら内部生殖器の進化に関する議論については扱わない．また，以下，各種名は基本的には学名表記とし，一般的に使用される和名がある種については初出時のみ和名を併記した．

表 1：キイロショウジョウバエ種群の 12 亜群（種亜群，species subgroup）とその代表種．代表種は本文中に登場するものや系統解析等の研究に用いられることが多いものを中心に選んだ．亜群より下の分類階級である種複合体（species complex）については，本文中に登場するもののみ示した

亜群名	代表種
1．*D. ananassae* 亜群	*D. ananassae, D. pallidosa, D. ercepeae, D. varians, D. atripex*, the *D. bipectinata* complex の 4 種（*D. bipectinata, D. parabipectinata, D. malerkotliana, D. pseudoananassae*）
2．*D. montium* 亜群	*D. baimaii, D. auraria, D. rufa, D. kikkawai, D. serrata, D. birchii, D. greeni*
3．*D. suzukii* 亜群*	*D. suzukii, D. biarmipes, D. mimetica, D. lucipennis*
4．*D. ficusphila* 亜群	*D. ficusphila*
5．*D. rhopaloa* 亜群	*D. rhopaloa, D. fuyamai*
6．*D. elegans* 亜群	*D. elegans, D. gunungcola*
7．*D. takahashii* 亜群	*D. takahashii, D. pseudotakahashii, D. lutescens, D. trilutea, D. prostipennis*
8．*D. eugracilis* 亜群	*D. eugracilis*
9．*D. melanogaster* 亜群	*D. erecta, D. orena, D. teissieri, D. yakuba, D. santomea*, the *D. melanogaster* complex の 4 種（*D. melanogaster, D. simulans, D. sechellia, D. mauritiana*）
10．*D. flavohirta* 亜群	*D. flavohirta*
11．*D. longissima* 亜群	*D. longissima*
12．*D. denticulata* 亜群	*D. denticulata*

*多系統とされる（本文参照）

2. ショウジョウバエ類の交尾器形態と用語体系をめぐる問題

本稿で議論の対象とするキイロショウジョウバエ種群も含めて，ショウジョウバエ科のオス交尾器は，phallic organとその周辺を取り囲む形で存在するperiphallic organの2つに大別して扱うと便利である．この区分は「交尾時にメスの生殖管内に挿入される部分」と「交尾時にメス生殖管とそれを囲むメス交尾器に外から接触・把握する部分」という機能的分類にほぼ相当する．ここではまず，*D. sechellia*（セイシェルショウジョウバエ；*D. melanogaster*亜群に属する）と*D. ananassae*（アナナスショウジョウバエ；*D. ananassae*亜群に属する）を例に，キイロショウジョウバエ種群のオス交尾器形態について概説する（図1）．メスの交尾器については，内部生殖器と併せて基本構造を図2に示す．本稿では基本的にMcAlpine（1981），Zhang and Toda（1992），Hu and Toda（2001）で提唱・使用されている用語体系に従う．これは，ハエ目（Diptera）全体での共通性が高い用語体系である．

2.1 オスのperiphallic organ

オスの交尾器の最も大きな部分を構成するのは第9腹節である．第9腹節背板（9th abdominal tergite）は鞍状に発達し，epandrium（図1a，b，d）と呼ばれる（多くの文献でgenital archとも呼ばれる；表2）．この後方背側には肛門が開口し，その左右両脇にはcercus（肛板）が存在する．種によっては肛板の特に腹側が強い剛毛等で修飾されている．その腹側で，epandriumの左右は細い架橋部で結ばれているが，そこから剛毛を備えた左右1対の突起が腹側へ向かって伸びており，これをsurstylus（把握片）と呼ぶ．一般に強い剛毛を多数備えるが，多くの種ではその一部は鈍端なpeg-like setae（ペグ状剛毛）となる．*D. ananassae*亜群の大部分の種などでは，cercusとsurstylusの間に，ventral cercal lobeと呼ばれるやはり剛毛を備えた1対の突起を有する場合がある（図1d）．Epandriumの左右腹側末端の突出部はventral lobeと呼ばれ，多くの長毛を備えるのが普通である．

2.2 オスのphallic organ

Phallic organは，hypandrium（第9腹節腹板が変形したものと考えられている）の背側に存在し，交尾の際以外はperiphallic organ，特に左右のsurstylusの間に隠れていて観察しにくい．後述する一部の例外を除き，中央に1本のaedeagusがあり，精巣で作られた精子や

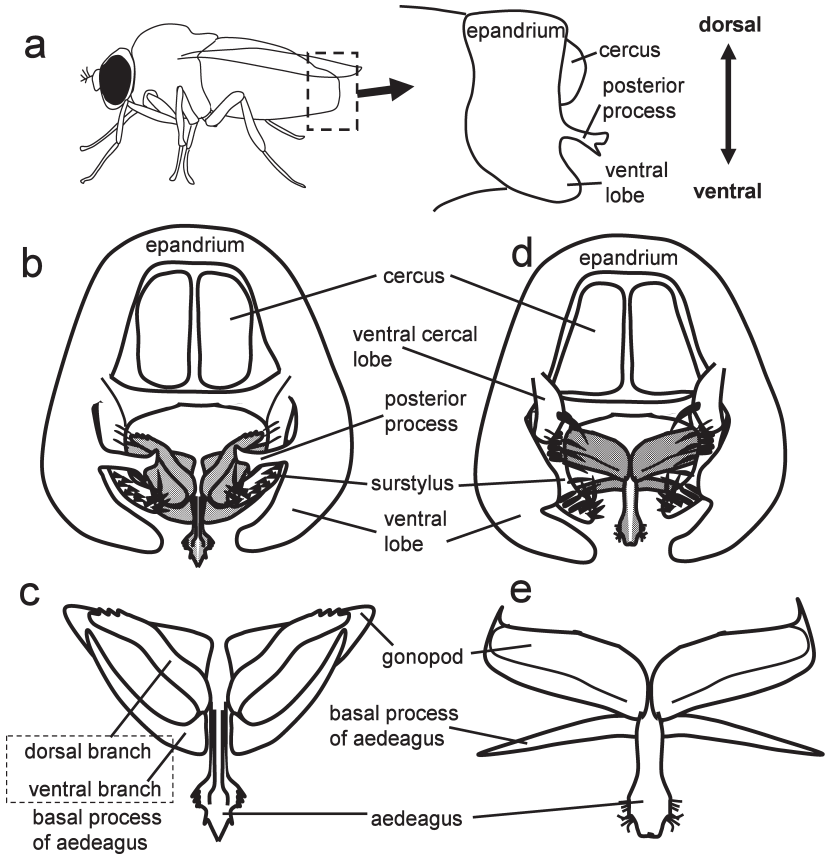

図1：キイロショウジョウバエ種群2種のオス交尾器の模式図．(a–c) *Drosophila sechellia*（*D. melanogaster*亜群），(d，e) *D. ananassae*（*D. ananassae*亜群）．(a)はオス側面図と交尾器部分の拡大図．(b–e) はオス交尾器を後方から見た図．(b，d) はperiphallic organとphallic organ双方を示し，後者は灰色で示している．(c，e) はphallic organの拡大図．Cercus等の毛や，phallic organの一部（hypandriumやparamere）は省略して描く．Phallic organは交尾時の開いた状態として描いてある．*D. ananassae*のphallic organについては図6も参照．

表 2：キイロショウジョウバエ種群の雌雄交尾器に関する用語

	本稿での用語	よく使われる別称
オス交尾器		
Periphallic organs		
	epandrium	genital arch, tergite IX
	cercus(-i)	anal plate(s), secondary clasper(s), tergite X
	ventral cercal lobe(s)	secondary clasper(s)
	surstylus(-i)	(primary) clasper(s)
	posterior process(es)	ventral lobe(s), lateral lobe(s), posterior lobe(s)
	ventral lobe(s)	lateral plate(s)
Phallic organs		
	hypandrium	sternite IX, novasternum
	hypandrial apodeme	ventral fragma
	aedeagus	penis, phallosome, phallus
	aedeagal apodeme	penis apodeme
	basal process(es) of aedeagus	
	dorsal branch(es)	
	ventral branch(es)	
	paramere(s)	anterior paramere(s)
	gonopod(s)	posterior paramere(s)
メス交尾器		
	oviscape	ovipositor
	oviscapt(s)	oviscape valve(s), ovipositor plate(s), ovipositor lobe(s), egg guide(s), vaginal plate(s), sternite VIII

付属腺で作られた化学物質を含む精液をメスへと渡す管として機能する．背側から aedeagus を囲むように 1 対の gonopod（多くの場合葉状）があり，交尾時には左右に開く．Aedeagus に対して腹側には，1 対の paramere と呼ばれる突起があり，数本の感覚毛（sensillum）を備えることで特徴づけられる．キイロショウジョウバエ種群では小さいことが多く（図 6 参照），交尾時の機能もよくわかっていない（ただし，3.2.1 において後述する *D. mimetica* など例外も多い）．Aedeagus の根元から関節して，もしくは aedeagus に融合して，通常 1 対の強く硬化した突起がある．この basal processes of the aedeagus（以下，basal process と略記）は，*D. melanogaster* 亜群においては 2 対あり，aedeagus の背側（dorsal branch）と腹側（ventral branch）に分けられる（図 1 c）．腹側の 1 対はしばしば gonopod と融合している．

2.3 メスの交尾器と内部生殖器

主に腹部の第 7 節以降から構成されるメスの交尾器は，一般にオスに比べて単純な構造をしている．ここでは内部生殖器と併せてその一般的特徴を概説する（図 2）．左右 1 対の卵巣で成熟した卵は輸卵管を通り，精子貯蔵器官である 1 対の spermatheca や 1 本の ventral receptacle の中に蓄えられていた精子によって受精される．受精卵はその後方にある uterus と呼ばれる部分に産卵まで保持される．ここは，メスの生殖管（reproductive tract あるいは genital tract）が太くなっている部分であるが，その後方で狭まり（post-vagina と呼ばれることがある；例えば Lung and Wolfner, 1999），腹端に外部生殖口として開口する．

外部生殖口は交尾の際にはオスの phallic organ の挿入を受けるとともに，産卵口として機能する．その部分は左右から oviscapt と呼ばれる sclerite に挟まれている．そのクチクラの硬化の程度は種ごとに様々である．これらの領域は産卵管に相当し，oviscape と呼ばれる（図 2）．

肛門はオスと同様に生殖口に対して背側に位置し，鞍状の第 8 腹節背板に囲まれた部分に開口する．第 10 腹節背板および第 10 腹節腹板がそれぞれ変形したものとされる epiproct，hypoproct の 2 枚の sclerite に上下から挟まれる形で開口しており，左右の cercus で挟まれているオスの場合とは異なる．メスの epiproct-hypoproct の部分は，交尾中にオス交尾器とかみ合うことは無いようで，一般に交尾器（genitalia）の範疇には含めないことが多い．

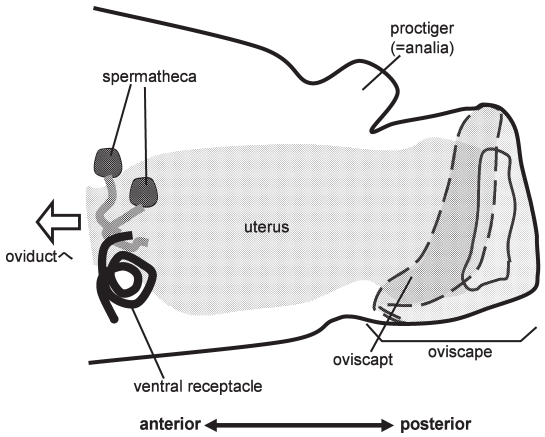

図 2：キイロショウジョウバエ種群のメスの内部生殖器および交尾器の模式図（側面図）．

2.4　用語体系をめぐる問題

以上概説したように，ショウジョウバエ類の交尾器形態，特にオスのそれは多くのパーツからなる大変複雑な構造であり，混乱や誤解の原因となっている（Kamimura and Polak, 2010）．問題をさらに複雑にしているのは，その用語体系の混乱である．ショウジョウバエ科以外のハエ目研究者が一般的に用いている用語体系（ただし，用語の統一や相同性に関する議論が全て解決しているわけではない）が存在するのに対し，モデル生物として長い歴史を持つショウジョウバエ類では，ショウジョウバエ研究者（例えば遺伝学者や発生生物学者）に特有の用語体系が複数あり，交尾器の同じパーツが研究者毎に違った名称で呼ばれているのが現状である（表2）．例えば，ventral lobe という同じ用語が，研究者によって periphallic organ の別の部位を指し示すのに使用されていることがわかる．

今後，ショウジョウバエ類のみならず，ハエ目の様々な分類群で分子発生学的な研究が進展し，交尾器形態の相同性や進化についての横断的な議論をおこなえる素地が形成されていくだろう．そのような状況を考えると，ハエ目全体での共通性が高い用語体系を採用していくことが望ましい．生態・行動分野等では，専門的な形態に関する用語よりも，「把握器」等の一般的な呼称の使用が好まれることもあるが，研究対象とした部位を図示するとともに，採用した用語体系とそれに基づく部位の名称を併せて明示することが重要であると考えられる（Kamimura and Polak, 2010）．

3．キイロショウジョウバエ種群の交尾器形態に関する進化・機能研究

3.1　交尾器形態の適応的意義に関する研究

交尾器の機能に関する研究は，そのアプローチから大きく二つに分けることができる．一つは特定の種類の交尾器の使用について記載的観察をおこなう，あるいは交尾器パーツに切除等の実験的操作を加えることにより，その機能を推定・検証するアプローチである．もう一方は，種内・種間にみられる交尾器形態の変異を利用し，その機能を推定する比較法のアプローチである．

代表的なモデル生物である *D. melanogaster* をはじめとして，ショウジョウバエ類はその種内の遺伝的変異（人為的に作出されたものを含む）に関する膨大な研究の蓄積がある．しかし，交尾器形態の遺伝的変異に関する研究は，発生生物学上のテーマに関わるものを除けば少ない．野生種の種内（あるいは生殖的隔離の認められない個体群間）に観察される交尾器形態変異の報告もあるが（例えば Matsuda et al., 2005; Joly et al., 2010），機能の研究とは結びついていないのが現状であり，形態的差異の比較に基づくアプローチによる研究は，全て種間の比較を通しておこなわれている．

3.1.1　種間比較に基づく研究

キイロショウジョウバエ種群の種間に見られる交尾器形態の差異に初めて説明を試みた論文は Robertson（1988）によるものと思われる．彼は，キイロショウジョウバエ種複合体（the *D. melanogaseter* sepceis complex）の4種，すなわち，*D. melanogaster* とそれに最も近縁な *D. simulans*（オナジショウジョウバエ），*D. sechellia*，*D. mauritiana* の3種を対象に，posterior process の種間の形態の違い（図3）に着目した研究をおこなっている．この突起は epandrium の一部が後方へ伸長したものであり（図1a，b），4種の共通祖先で進化したと考えられている（Jagadeeshan and Singh, 2006）．最も簡便かつ確実にこの4種を識別するための形質として利用されており，種間雑種を利用した研究から，その形態差の遺伝学的側面については研究の蓄積がある（Coyne, 1983; Coyne and Kreitman, 1986; Liu et al., 1996; True et al., 1997; MacDonald and Goldstein, 1999; Zeng et al., 2000）．しかし，機能の観点から探求した研究は，Robertson（1988）までなかった．彼は液体窒素で固定した交尾中のペアを走査型電子顕微鏡（以下，SEM と略記する）で観察し，① posterior process は，交尾中にメスの腹節第7および第8背板（Robertson, 1988 では第8および第9背板となっているが，これは退化したとされる腹節のカウントの仕方の違いによるものである）の下に隠されるかたちで，メスの腹部を把握し，②メスにおいて，posterior process の挿入を受け入れると考えられる部位には，posterior process のサイズの種間差に相関した違いが観察される，と報告している．そして，大きな posterior process を持つ *D. simulans* のオスと *D. mauritiana* のメスでは種間の交尾が成立し難く（48時間以内の交尾成功率0％），逆では高率（76％）で交尾が成功することの原因を，この雌雄の交尾器形態のサイズの対応にあるものと考察している（Robertson, 1988）．

上記①の posterior process の機能に関しては，後述の通り，その後の研究において同様の観察結果が得られている．しかし，②の雌雄の交尾器に見られる相関については，その後，この結果を追認する報告は無い．ハエ類の腹部は，硬化した背板・腹板が，柔軟な節間膜に

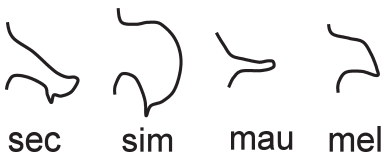

図3：キイロショウジョウバエ種複合体（the *Drosophila melanogaster* species complex）4種の posterior process の形態．左側のものを後方から見た図．sec，*D. sechellia*; sim，*D. simulans*; mau，*D. mauritiana*; mel，*D. melanogaster*．図1a，bも参照．

よって結合された蛇腹状の構造であり，彼の記載した部位は再現性の高い観察をすることが困難である（図 4）．また Robertson（1988）には詳細な観察手法の記載がなく，追試をおこなうこと自体が困難となっている．

Eberhard and Ramirez（2004）は，キイロショウジョウバエ種群の 2 種（*D. melanogaster* と *D. malerkotliana*）と，他の 2 種群に属する各 1 種（*D. saltans* と *D. willistoni*）について，オスの交尾器の種間差を探る研究をおこなっている．ここでは後者 2 種の観察結果の詳細については省略する．彼らは，交尾中のペアを液体窒素で固定し，冷凍状態を保ったまま，エタノール固定と乾燥をおこない，SEM での観察をおこなっている．一部については，固定後に雌雄を引き離し，交尾中に隠されて見えない部分の観察もおこなっている．観察にあたり，交尾器形態，特にオスのそれの速い進化に関して次の三つの主要な仮説を挙げ，それぞれの仮説が成立した場合の予測を与えている．

交尾器の進化が同種の識別のための「鍵と鍵穴」として進化する場合（Shapiro and Porter, 1989），雌雄交尾器の間には形態的な対応が予測される．雌雄が交尾の成立や精子の授受に関して対立状態にある時（性的対立 sexual conflict と呼ぶ），例えば，オスは交尾を望むのに対して，メスが交尾を拒否しようとする際，雌雄交尾器の形態に軍拡競争（arms race）が生じると予想される．この時，例えばオスが交尾を拒否しようとするメスを把握するような構造を進化させるのに対し，メスはその把握を逃れるような構造を進化させるだろう．これは拮抗的共進化（antagonistic coevolution）仮説と呼ばれ，「鍵と鍵穴」仮説と同様に雌雄交尾器の間に形態的対応が見られることが予測される．一方，cryptic female choice 仮説は，メスはオスの交尾器形態に基づいて，そのオスから受け取る精子の量や受精の確率を変化させているという考えである．オスの交尾器形態と，そのオスの精子によって受精された子の生存率や繁殖率との間に相関がある場合，メスによる精子の選択が進化しうることが理論的に示されている（Yasui, 1997）．先の「鍵と鍵穴」仮説が同種か異種かの識別に関わる仮説であるのに対し，この仮説は同種オス間での選択である点が異なる．この仮説が成立する場合，メスは感覚神経系を介して選択をおこなっている可能性もあるため，雌雄交尾器の間の形態的対応が観察できるとは，必ずしも予測されない．

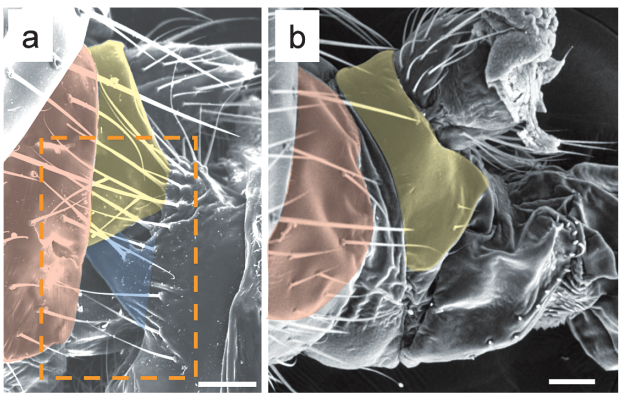

図 4：(a)交尾中，および(b)伸展された状態での *Drosophila sechellia* のメスの腹端部の SEM 像．赤，黄色で示された部分はそれぞれ第 7 および第 8 腹節背板を示す．(a)において，青で示されているのは第 7 および第 8 腹節背板の下に挿入されているオスの posterior process，破線で囲まれている部分が Robertson（1988）の Fig. 2 において，オスの posterior process の種間差（図 3 参照）に対応する構造として描かれている部分である．スケールバーは 25 μm．

彼らの観察の結果は次の通りである．*D. melanogaster* の posterior process（Eberhard and Ramirez, 2004 中では ventral lobe）に関しては，Robertson（1988）同様，主にメスの腹部第 7 背板の下に隠されることを報告している．観察した 4 種すべてについて，左右の surstylus は交尾中に開いていることを観察し，メスの oviscapt（Eberhard and Ramirez, 2004 中では oviscape valve）を押し開き，aedeagus 等の挿入を助ける働きを持つと考察している．*D. malerkotliana* の 1 対の basal process（Eberhard and Ramirez, 2004 中では aedeagus が左右に分割されたものと誤認されている）については，左右が融合した状態で通常の aedeagus と同様に機能すると報告されているが，これは表面構造のみの観察に基づいたことによる誤りであり，本種を含むフタクシショウジョウバエ種複合体（the *D. bipectinata* complex）の特殊な交尾については 3.2.2 において詳述する．

彼らはまた，メス交尾器の中で数少ない硬化した構造である oviscapt に，オスの交尾器形態の種間差に対応した点を探した．しかし，そのような変異をメス側に見出すことができなかった彼らは，「鍵と鍵穴」仮説や拮抗的共進化の証拠は得られなかったと結論付け，消去法による弱い状況証拠ではあるが，メスの感覚神経系を介した cryptic female choice がショウジョウバエ類の交尾器進化に重要な役割を果たしているものと推察している．

Jagadeeshan and Singh（2006）は Robertson（1988）と同様にキイロショウジョウバエ種複合体における posterior process の種間差（図 3）に着目した研究をおこなっている．その手法は Eberhard and Ramirez（2004）が用いたものと基本的に同様であるが，特に *D. melanogaster* と *D. simulans* の 2 種について，種間交尾も含めて，交尾の時間経過に沿った固定をおこない，交尾経過中の雌雄交尾器の変化を報告している．Robertson（1988）や Eberhard and Ramirez（2004）の観察とほぼ同様の結果を報告しているが，cercus がメスの oviscapt 基部の把握に用いられている可能性を新たに指摘している．また，種間交尾においては，種内で見られたような交尾器のカップリングの成立が遅れるものの，交尾時間についてはオスが決定する傾向にあ

り，種内交尾と大きな違いがないことを報告している．この研究でもまた，メスの側にオスの種間差に対応する構造は見出されていない．

Kamimura（2010）も *D. melanogaster* の交尾を観察し，posterior process について同様の観察結果を得ているが，これについては関連した話題とともに 3.2.1 において後述する．

3.1.2　交尾器形態の実験的操作に関する研究

Polak and Rashed（2010）は，マイクロレーザーを用いて，*D. bipectinata*（フタクシショウジョウバエ）の ventral cercal lobe（図 1 d）の先端の剛毛を切除する実験を行い，手術を受けた個体の交尾成功率および交尾に成功した際の精子の受け渡しの有無を調査した．その結果，コントロール（周辺の他の剛毛をランダムに選んで切除したもの）に比べ，交尾成功率が半減するが，交尾に成功した場合には，コントロールと変わりなく精子を渡していることを明らかにした．このことは，ventral cercal lobe が把握器官の一つとして重要な役割を持つことを示唆している．彼ら自体は，この器官の使用を直接観察していないが，同様な交尾器の構造を持つ *D. malerkotliana* の交尾を観察した Eberhard and Ramirez（2004）の Figs. 6, 9 には，ventral cercal lobe がメスの oviscapt の腹側と接触している様子が写されており，推定された把握機能と矛盾しない（Kamimura and Polak, 2010）．ただし，Eberhard and Ramirez（2004）ではこの点について言及されていない．

同様に，Acebes et al.（2003）によって，*D. melanogaster*，*D. simulans*，*D. sechellia* の surstylus 先端付近の感覚毛をピンセットで切除する実験がおこなわれている．*D. melanogaster* では，片側一方のみの切除では交尾成功率に有意な低下は認められないが，左右両側を切除すると交尾成功率が半減すると報告されている．ただし，片側のみの切除の場合，オスの交尾姿勢が感覚毛を切除した側の反対側に傾く．このような効果は，他の 2 種では認められていない．また，ventral lobe の毛を同様に処理した場合には交尾成功率や交尾姿勢に影響は無かった．この研究では，精子がメスに渡されているか否かについては調査されていない．

3.2　交尾時のオス交尾器による創傷現象

交尾の際にオスが交尾器により，メスの交尾器やその周辺に傷をつける現象が，甲虫類（Crudgington and Siva-Jothy, 2000; Rönn et al., 2007），アリ類（Baer and Boomsma, 2006; Kamimura, 2008），クロバエ科（Merritto, 1989）・ツヤホソバエ科（Blanckenhorn et al., 2002；Teuschl et al., 2007）といったハエ類など，様々な分類群の昆虫で報告されている．そのような現象を本稿では交尾時創傷（copulatory wounding）と呼ぶことにするが，キイロショウジョウバエ種群でも広く生じていることが分かってきた（Kamimura, 2007, 2010）．交尾時創傷についてこれまでにおこなわれてきた研究は，その種間変異に関するものであるが，ここでは 3.1.1 と分けて，研究例を紹介したい．

3.2.1　キイロショウジョウバエ種群における交尾時創傷

これまでにキイロショウジョウバエ種群 9 亜群 28 種について調査した結果，82%にあたる 23 種において，交尾したメスの交尾器や生殖管の末端部に現れる褐色斑が観察されている（Kamimura, 2007, 2010）．昆虫は体表に傷が生じた場合，免疫反応により傷の部分に「かさぶた」に相当する構造が作られ，褐色を呈するようになる（Pathak, 1993）．既交尾のメスにのみ観察される「かさぶた」は，交尾時創傷を示唆するが，その位置，サイズ，数は種によって，場合によっては種内においても変異が見られる（Kamimura, 2007, 2010）．いくつかの種では傷を負わせるオス器官が特定されている．*D. mimetica* では，オスは鋭くとがった 1 対の paramere を持ち（図 5 a），メス交尾器の背側体表（oviscapt と analia の間の部位）に傷を負わせる．メスが傷を負う部分は 1 対の椀状の窪みとなっている（図 5 b-d；Kamimura, 2007）．*D. melanogaster* のメスにも交尾時創傷が見られるが，本種の場合はオスの aedeagus に付属する 2 対の basal process のうち，背側の 1 対（dorsal branch）によって傷が作られる（Kamimura, 2010）．この突起は，aedeagus とともに交尾時にメスの生殖管内に挿入されるため，*D. mimetica* とは異なり，生殖管の表面，いわばメスの「内表面」に傷がつけられる（Kamimura, 2010）．メスの傷を負う部分には，ひ

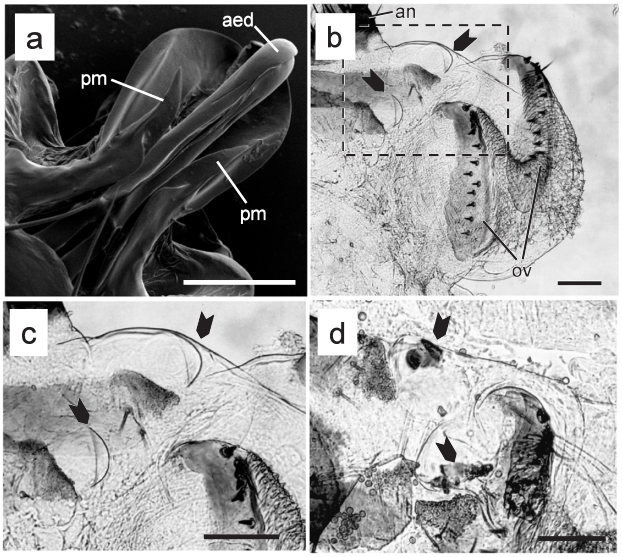

図 5：*Drosophila mimetica* の雌雄交尾器．(a) オスの phallic organ には aedeagus (aed) の両側に左右 1 対の鋭い先端をもつ paramere (pm) がある．(b) メス交尾器には analia (an) と oviscapt (ov) の間の背側体表に 1 対の椀状のポケット（矢印で示す）を持つ．(c) 未交尾メスにおける (b) の点線部の拡大．(d) 既交尾メスの (c) と同一部；オスの paramere によって生じた傷（矢印）がポケットに生じている．スケールバーはすべて 50 μm．

だ状の浅い窪みがあり，そこに dorsal branch が交尾中にかみ合うことで，dorsal branch 先端により傷がつくものと考えられている．また，この創傷部位近傍は，メスの体表側からはオスの posterior process によって把握される．すなわち，生殖管の内外から挟みこむ形で，交尾中のメスが把握されている（Kamimura, 2010）．このように，交尾時にメスが傷を受ける部位や，創傷に関与するオスの構造は多様である．以下，創傷の特別なケースである traumatic insemination（傷穴を通して精子を渡す現象）を紹介し，交尾時創傷の進化に関する議論と研究の現状を概観したい．

3.2.2 傷穴を通して精子を渡す（traumatic insemination）

フタクシショウジョウバエ種複合体（the *D. bipectinata* species complex）は，*D. bipectinata*，*D. parabipectinata*，*D. malerkotliana*，*D. pseudoananassae* の 4 種からなり，*D. ananassae* 亜群に属する．この 4 種に近縁な *D. ananassae* の phallic organ が 1 本の aedeagus，1 対の basal process，1 対の paramere，1 対の gonopod からなるのに対して（図 1 e，6），フタクシショウジョウバエ種複合体の 4 種では，中心の aedeagus が著しく退縮する代わりに，その部分に 1 対のよく発達した爪状突起を持つ点が特殊である（図 6）．この爪状突起は aedeagus が二叉したものと従前は解釈されてきた（例えば Bock and Wheeler, 1972; Eberhard and Ramirez, 2004）．しかし，SEM 観察等の結果，フタクシショウジョウバエ種複合体の aedeagus は退化して無色透明の柔軟な膜状構造になっており，2 本の爪状突起はその両側の basal process が大きく発達したものであった（Kamimura, 2007）．この特異な交尾器構造を持つグループの精液移送の過程を明らかにするため，交尾中のペアを瞬間冷凍固定し，共焦点レーザー顕微鏡で観察をおこなった．この際，オスに蛍光色素（Rhodamine B）を摂食させることで精液を経口的に染色した．その結果，フタクシショウジョウバエ種複合体の basal process はメスの生殖口の両脇にある膜質のポケット（窪み）に挿入され，その底を破り，傷穴を通して精液を渡すことが明らかとなった．近縁な *D. ananassae* では，basal process はやはりメスにある同様のポケットに傷を与えるが，精子は傷穴ではなく，生殖口に挿入された aedeagus から渡される（Kamimura, 2007）．

このような特殊な精子の渡し方の進化をもたらした選択圧については不明である．創傷行動そのもののオスにとっての意義は以下で議論するが，メスにとってはコストである可能性が高い．メスは繁殖のために最低でも 1 回の交尾から精子を得る必要があるが，その必須の移精（sperm transfer）と創傷とを不可分とすることで，オスがより確実にメスに傷を与えている可能性がある．詳しい精液移送のメカニズムの解明とともに，今後の研究課題である．

傷穴を通して精子を渡すという現象はネジレバネ類や一部のカスミカメ類などいくつかの分類群の昆虫で報告されているが，特に研究されているのはトコジラミ類である（例えば Siva-Jothy, 2006）．興味深いことに，トコジラミ類においても創傷に用いられる器官は aedeagus（カメムシ類において一般的に精子を渡す器官）そのものではなく，それに隣接する paramere と呼ばれる突起が発達し，aedeagus と融合したものである（Siva-Jothy, 2006）．Traumatic insemination の進化的起原についてはほとんど情報がないが，このような類似性は，その起原が交尾時の創傷現象であった可能性を示唆する．

3.2.3 精液中化学物質の生理的作用との関連

交尾時創傷がキイロショウジョウバエ種群の多くの種で観察されることがわかってきたが，その行動のオスにおける意義やメスに対する影響についてはほとんど何も明らかにされていないのが現状である．Siva-Jothy

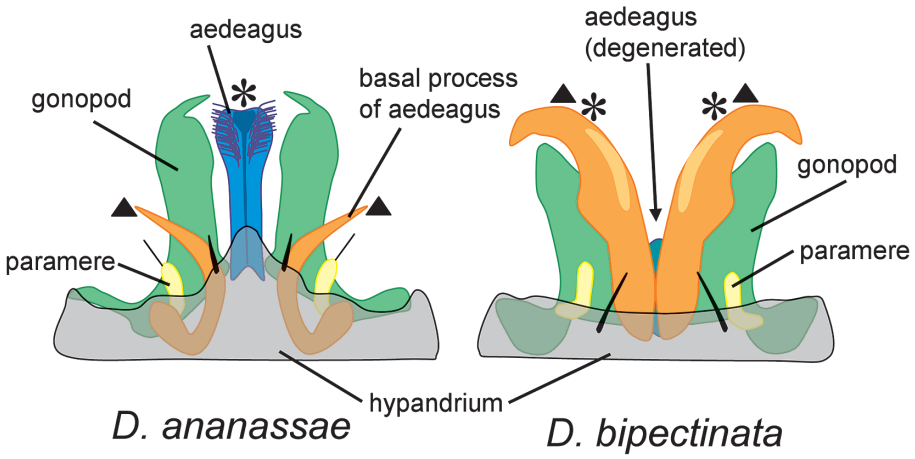

図 6：*Drosophila ananassae* と *D. bipectinata* の phallic organ の比較．腹側（ventral）より観察した図．＊はメスに精子を渡すのに使用される器官を，▲は創傷器官を示す．*D. bipectinata* の場合は 1 対の basal process が両機能を兼ねている．*D. ananassae* の phallic organ は，図 1 d，e とは異なり，閉じた状態で描いてある．

(2009) はこのような交尾時創傷行動の意義として，オスの生産する精液物質のメス体内への浸入口としての機能を提案している．*D. melanogaster* をはじめとする同種群の多くの種において，オスが交尾相手のメスの生理特性を自分に有利な方向に変化させる精液タンパク質を生産することが報告されている（最近の総説としては Ravi Ram and Wolfner, 2007 参照）．これらのタンパク質はオスの精巣付属腺で生産されるため，accessory gland protein (s) を略し Acp (s) と呼ばれることが多い．その具体的作用は，その後の交尾拒否行動，速やかな排卵・産卵などであるが，同時にメスの寿命や生涯産卵数を低下させる「毒」としての作用も報告されている．このようなことから，メスが再交尾する前に，自分の渡した精子による受精卵を多数産ませるという，オスによる化学物質を介したメスの行動制御（ケミカルコントロール）と解釈されている．

Lung and Wolfner (1999) は，キイロショウジョウバエにおいて各種 Acp がオスからメスへ渡された後のメス体内での分布を調査した．一部の Acp はメスの内部生殖器に局在するのに対し，他の Acp，特に分子量の小さいものはメスの体液中からも検出されることがわかった．また，免疫染色の結果から，この体液への移動はメス生殖管の post-vagina 領域で生じているものと推測されている (Lung and Wolfner, 1999)．Post-vagina 領域は，キイロショウジョウバエにおいてオスが傷をつける部分であるが，もしこの傷が Acp の侵入口として機能している場合，Lung and Wolfner (1999) が観察したような，分子量依存的な浸入が起こるとは考え難い．実際に，先に紹介した手法で精液を蛍光染色し，傷から体腔への精液侵入を調査したが，そのような様子は観察されなかった (Kamimura, 2010)．これは傷が aedeagus 先端の射精口に対して後方に生じるため，精液と接触することが少ないためと考えられた．このようなことから，*D. melanogaster* においては，Acps の侵入口を作るために交尾時創傷が進化してきたとは考え難い (Kamimura, 2010)．同様に，*D. mimetica* のように外部体表にのみ傷をつける種類でも傷は精液の侵入口になりえない．しかし，*D. eugracilis* (*D. eugracilis* 亜群) では，射精口周辺に多くの棘状突起を持ち，それによってメス生殖管に傷が生じ，そこから精液が体腔に侵入する様子が観察されている (Kamimura, 2010)．このような種類においては，Siva-Jothy (2009) の仮説を実験的に検討していくことが重要であろう．

3.2.4　物理的ダメージや感染リスクとの関連

交尾時創傷行動の，オスにとっての適応的意義に関するもう一つの仮説として以下のようなものがある．創傷による物理的ダメージにより，①傷のためメスが再交尾することのコストが大きくなり，再交尾が抑制される (Johnstone and Keller, 2000)，または②メスの期待余命が短縮し，それに対する適応的応答としてメスがより早くに（再交尾前に）多くの卵を産卵するようになるため，交尾時に創傷したオスの適応度が上がる（例えば Lessells, 1999）という考えである．これは交尾をめぐる性的対立にもとづく仮説であり，ここでいう物理的ダメージには，傷口を通しての病原体への感染リスクも含めて考えることができる．これら仮説の検証実験は交尾時創傷が発見される以前に，Morrow et al., (2003) により *D. melanogaster* について既におこなわれていた．

Morrow et al. (2003) は，*D. melanogaster* を含む 3 種の昆虫類について，人為的な創傷がメスの繁殖に及ぼす影響を調査した．具体的には，メスの触角・肢・翅のいずれかを一つ切除する，あるいは胸部または腹部を細い針で刺すという 5 種の物理的ダメージを与えたうえで，その後の産卵速度と再交尾率を調査している．結果として，*D. melanogaster* では，胸部・腹部への創傷により産卵速度は低下しても，再交尾率は変わらず，オスに「交尾終了後の利益」をもたらすものではないと結論している．この結論は，彼らが調べた他の 2 種の昆虫についても同様であり，交尾中にメスにダメージを与える行動は，例えば交尾の際に拒否しようとするメスを把握する際の副産物であり，ダメージそのものには機能がないものと考察している．

Morrow et al. (2003) の実験において人為的につけられた傷は，交尾による傷と部位も程度も異なるが，*D. melanogaster* においては交尾による傷の方がより軽微であると考えられ，交尾時創傷がオスの「交尾終了後の利益」に貢献している可能性は低いと考えられる．ハヤトビバエの 1 種など他の昆虫でも，交尾時創傷は交尾中の把握による副産物という見方を支持する結果が報告されている (Edvardsson and Tregenza, 2005; Teuschl et al., 2007; Hotzy and Arnqvist, 2009)．

交尾時創傷によって，メスが病原体に感染するリスクが高まることも予想されている (Siva-Jothy, 2009)．*D. melanogaster* においては，交尾の際にオスからメスに渡される Acp の作用によって，各種抗菌タンパク質の転写活性の上昇が報告されており（例えば Domanitskaya et al., 2007)，最近では，病原体の侵入を伴わない創傷のみでも，一部の抗菌タンパク質が発現誘導されることが明らかとなってきている (Wigby et al., 2008)．メスに対する物理的ダメージの影響は，このような免疫防御によってマスクされている可能性がある．抗菌活性の進化も考慮に入れて，交尾時創傷の進化的意義を検討していく必要があるだろう．

4．今後の研究への展望

4.1　旧来の研究の問題点

ここまで，キイロショウジョウバエ種群に観察される

雌雄交尾器形態の変異とその進化にまつわる研究事例を紹介してきた．同種群の研究に対象を絞ったが，ここで紹介したような機能研究は，ショウジョウバエ類の他のグループでは，筆者らの知る限りおこなわれていない（ただし，Eberhard and Ramirez, 2004 も参照）．ショウジョウバエ類の多様な交尾器形態は，飼育・入手の容易さや遺伝・系統に関する情報の豊富さから，交尾器形態の多様化・進化の機構を探る格好のモデルとなり得る可能性を秘めている．しかし，その交尾器の進化のメカニズムについては多くの点が推察の域を出ていないのが現状と言えるだろう．各交尾器パーツの機能に関してさえ，交尾時にどこにどのような形で位置するのかといった基本的な情報が不明であったり，誤って認識されていたりする．そのような情報のないままに，切除実験や種間比較をおこなったとしても，結果を適切に解釈することは困難である．オスの交尾器は複雑であり，メス交尾器は柔軟で形が定まり難く，交尾時に多くのパーツが外から見えなくなるような昆虫の交尾器に対して，新しい観察手法が必要である．以下我々が検討している交尾中ペアの透明化の手法を紹介し，その可能性とともに今後の展望を議論したい．

4.2　透明化手法の開発と検討

McGurk et al.（2007）はショウジョウバエの外皮を透明化し，内部構造を 3 D で観察する手法を考案している．McGurk et al.（2007）は交尾器や交尾中のペアの観察をおこなっていないが，以下，我々がその手法を交尾中ペアに応用した結果を紹介する．

交尾時の雌雄交尾器の位置関係を保って観察するためには，ペアの瞬間固定が必要となる．そのためには液体窒素（Eberhard and Ramirez, 2004 などで使用されている）もしくは粒状ドライアイスを加えた 100%エタノールを，交尾中のペアの入った容器に注ぐことで固定できる．そのままエタノール中で−20°Cに保管し，2 週間以上固定することで，常温に戻しても交尾中の雌雄交尾器の位置関係が保たれる．

充分に固定した交尾ペアを 10 分程度，常温の水中に移すことで，次の寒天包埋の前処理とする．1 %寒天中に包埋し，寒天が固まったらトリミングをおこなう．切り出したブロックを 100%メタノールもしくは 100%エタノール中で脱水する．脱水が充分でないと次の処理において，寒天が半透明に硬化してしまい観察が困難となるので，ブロックが大きい場合は特に注意が必要である．BABB 溶液（ベンジルアルコールと安息香酸ベンジルを 1：2 v/v で混合した溶液）に寒天ブロックを移すと，透明化および脱色が始まる．BABB 溶液で透明化・脱色をおこなう最適な時間は用いる材料と観察したい構造に依存する．*D. bipectinata* のように，雌雄の腹部末端に黒色部が少ない種では，1 日以内に phallic organ を透視できるようになる（図 7）．*D. melanogaster* のように特にオスの腹部末端の黒化部が広い種であっても，100 日程度の液浸により全身がほぼ無色透明になる．内部の構造も同時に脱色されていき，コントラストの弱い構造は観察が難しくなっていくため，定期的に取り出して検鏡し，最適な処理時間を求める必要がある．

4.3　おわりに

ショウジョウバエ科，そしてハエ目に限らず，これまでの昆虫類の交尾器形態の研究はメス側の研究が立ち遅れてきた．メス側の交尾器には変異が少ないということが言われてきたが，ここで紹介してきた研究事例のように，オス交尾器に対応するメス側の形態変異は柔軟な膜質構造上に生じることが多く，従来の乾燥処理や SEM による表面構造の観察のみではそれらを特定することが難しい．本稿で紹介した透明化の手法は，交尾時の雌雄交尾器の対応を位置関係を保ったまま観察できるため，柔軟で着色部の少ないメス交尾器において，着目すべき部位の特定を可能としてくれる．

ここに紹介したキイロショウジョウバエ種群の研究結

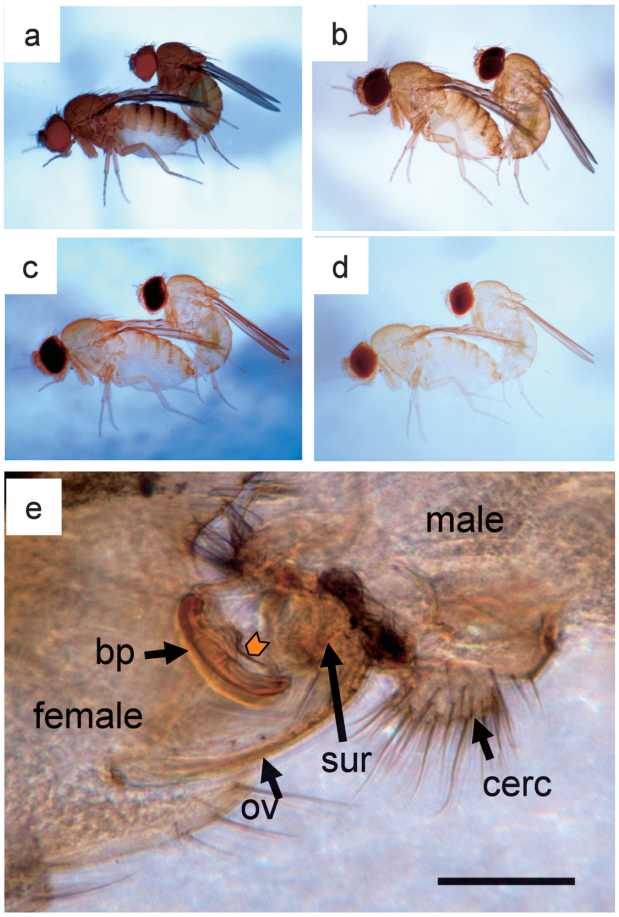

図 7：交尾中に固定された *Drosophila　bipectinata* ペアの BABB 処理による透明化の経過．(a) BABB 処理前の寒天包埋サンプル，(b) BABB 処理 1 日後，(c) BABB 処理 14 日後，(d) BABB 処理 28 日後，(e) 透過により包埋状態のまま観察可能となった雌雄交尾器のカップリング（cerc, cercus; sur, surstylus; bp, basal process; ov, oviscapt）．矢印（橙色）の先に，メスの膜質のポケットの一部が見える．スケールバーは 100 μm．

果から，本種群のオスは交尾中のメスを把握するための様々な構造を発達させていることがうかがえる．なぜ把握のための構造を複数持つのか，それらの構造は把握以外の機能を持ち合わせていないかなど，これらの点を解明するためには，複数の手法を駆使した多角的な検討が必要となるであろう．

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Fluctuating asymmetry in *Drosophila*

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Fluctuating asymmetry (FA) or subtle differences between left and right sides of a trait has been considered as an indicator of an organism's ability to cope with genetic and environmental stresses during development. It is considered to play an important role in mate choice. However, over four decades of work on various organisms such as insects, reptiles, birds and mammals has left the field with no clear-cut relationship between increased fluctuating asymmetry and stress due to inconsistency in the results. Despite this, fluctuating asymmetry is still assumed to indicate developmental instability caused by various perturbations (either genetic and/or environmental in origin) during development. The present article provides a unified framework for the better understanding of fluctuating asymmetry and its association with stress in particular reference to *Drosophila*.

Introduction

The temptation to understand the patterns of asymmetries dates back to Aristotle, who observed the regular patterns of bilateral asymmetries in animals likes crabs, lobsters and crayfishes (Herrick, 1909). Earlier, it was thought that the asymmetries (i.e., deviations from symmetry) are unimportant, but recently researchers have begun to realize how important these asymmetries are (Palmer, 1996), as they play an important role in evolutionary patterns and processes (Wu et al., 2007). Bilateral symmetry (a pervasive feature of the body plans of most animals) gets disturbed in the presence of genetic and environmental stresses, and as a result, either developmental noise increases or developmental stability decreases (for definitions, see Table 1). Therefore, evolutionary biologists have started using asymmetry as a measure of developmental homeostasis (Table 1). The rationale behind this is that the same genetic and developmental programs control the development of the left and right sides of a bilaterally symmetrical organism and thus (any) departures from perfect bilateral symmetry can be used as a measure of genetic or environmental perturbations (Batterham et al., 1996).

The deviations from perfect symmetry can be conveniently grouped into three categories: directional asymmetry (DA), antisymmetry (AS) and fluctuating asymmetry (FA) (Van Valen, 1962). DA occurs when there is normally a greater development of a character on one side of the plane of symmetry than on the other (Van Valen, 1962), whereas AS occurs when most of the individuals in a population are asymmetric but it is unpredictable which side of an organism shows greater development (Timofeef-Ressovsky, 1934; Graham et al., 1993a). FA is defined as minor random deviations from perfect bilateral symmetry (Ludwig, 1932; Van Valen, 1962). The three different asymmetrical patterns are widespread throughout the natural populations (Polak, 2003). Nevertheless, under stressful conditions, there are many examples for the transitions in these three forms of asymmetry (i.e., from AS to DA, DA to FA, and also FA to AS) (Mather, 1953; McKenzie and Clarke, 1988; Graham et al., 1993a; Smith et al., 1997; Klingenberg et al., 1998; Lens and Van Dongen, 2000; Schneider et al., 2003; Leamy and Klingenberg, 2005). Out of three kinds of asymmetry, FA is used to measure developmental instability as it arises due to the random variations causing a structure to depart from its ideal symmetry during development, whereas antisymmetry and directional asymmetry are believed

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Table 1 : An overview of concepts used in the article

Terms	Explanations
Developmental noise	Any uncontrollable variation in phenotype due to random events during development
Developmental homeostasis	The property of an organism to adjust itself to variable conditions during development
Developmental stability (DS)	The ability to buffer development against random perturbations of environmental or genetic origin
Developmental instability (DI)	Random perturbations of environmental or genetic origin affecting development along developmental trajectory within particular environment
Fluctuating asymmetry (FA)	Small random deviations from perfect bilateral symmetry, i.e., normal distribution of right minus left differences (R-L) about a mean of zero
Directional asymmetry (DA)	When there is normally a greater development of a character on one side of the plane of symmetry than on the other, resulting in normal distribution of R-L differences about a mean which differs significantly from zero
Antisymmetry (AS)	When one side of the plane is consistently larger than the other, but the larger side may be either right or left at random, resulting in a bimodal distribution of R-L differences about a mean of zero
Positional fluctuating asymmetry (PFA)	Differences in the placement of bristles on an area, e.g., sternopleuron, between the two sides of the body, and thus as a measure of bristle positions or shape differences between the two sides

to have unknown genetic bases, and therefore are not commonly seen as reflective of developmental perturbations (Palmer, 1996).

The investigations on FA are burgeoning rapidly and are also attracting many *Drosophila* workers, and therefore, an extensive review is required to update the recent advances in this field. In view of this, an attempt has been made to include all the major investigations on fluctuating asymmetry in *Drosophila* as well as to discuss the role of FA as an indicator of stress and individual quality in sexual selection.

1. Fluctuating asymmetry

The term 'fluctuating asymmetry (FA)' is coined by Wilhelm Ludwig in his monumental monograph on biological asymmetry in 1932. FA is defined as 'small random deviations from a prior expectation of symmetric development in morphological traits' (Ludwig, 1932). Interest in FA stems from the notion that there is a target state of symmetry which all individuals aim to achieve, and those individuals who fail to develop symmetrically are, in some way, of poorer quality than those that do achieve symmetry. The core idea of FA studies is that the corresponding parts on the left and right sides of a bilaterally symmetrical organism are separate replicates of the same structure. The body sides of an individual share the same genome and constant environment, and also the external effects on development will be the same on both sides. Therefore, any deviation from symmetry is the consequence of an inability to cope with environmental or genetic stress (Tomkins and Simmons, 2003). Therefore, fluctuating asymmetry has been considered to be a putative general biomarker of stress, as it responds to a wide range of stresses, and also has been correlated with many life history traits (Leung et al., 2003). Nevertheless, the literature has been plagued by controversy and heterogeneous results (e.g., Møller, 1997; Clarke, 1998; Palmer, 1999). Despite this, the most accepted view appears to be that FA is an indicator of stress and mate choice (Swaddle, 2003; Leamy and Klingenberg, 2005).

FA can be measured both at the population- and individual-levels. At the population level, it is usually measured as the variation of differences in the size, length and/or count of bilateral structures between the right and the left sides (R-L). At the individual level, it is most often assessed by mean of unsigned left- minus right-side differences (|R-L|). However, there are other indices of FA also (for details see Palmer, 1994). The analysis of FA is usually affected by measurement error, other types of asymmetry (DA or AS) and trait size. The appropriate statistical procedures for checking measurement error in the data and for adjusting DA, AS and size effects on FA and for testing significant differences in FA among two or more groups are discussed in detail by Palmer and Strobeck (1986, 2003).

2. Fluctuating asymmetry as an indicator of stress

Fluctuating asymmetry (FA) has been widely used

as an indicator of genetic (Table 2) and/or environmental stress (Table 3). The levels of FA are generally compared in populations that are subjected or not subjected to stress (Leamy and Klingenberg, 2005). The hypothesis in such comparisons is that FA will be higher in the more stressed populations compared to the control or unstressed populations (e.g., Pankakoski et al., 1992; Graham et al., 2000; Imasheva et al., 1997a, b; Rasmuson, 2002; Polak, 2003; Leamy and Klingenberg, 2005). In support of this approach, there are several studies that have detected FA responses to stress in different species of *Drosophila*. However, on the other hand, there are also many studies that have been unable to detect the expected relationship between stress and FA (see Tables 2 and 3). The inconsistency in the results depends on the degree of stress imposed, choice of trait and FA indices (Hoffmann and Woods, 2003; Leamy and Klingenberg, 2005). Moreover, in the studies dealing with the environmental stress, it is often assumed that FA has no genetic basis and the differences in FA levels of the stressed and unstressed populations are purely environmental in origin. But the levels of FA may also show some genotypic differences (unless isogenic lines or genetically identical lines are used) in response to stress. Similarly, for the studies dealing with genetic stress, the environmental conditions should be controlled enough so that the results of FA analysis should not be under the influence of internal and external components of the environment (Zhang and Hill, 2005). There is a report that the levels of FA for different morphological traits vary significantly among laboratory populations of *D. ananassae* (which were reared in controlled laboratory conditions, i.e., food, humidity and temperature, for several generations). The variation in the levels of FA among the laboratory populations is thought to be under the influence of internal component of the environment and genetic variability of populations as they were collected from different ecogeographical localities of India (for details, see Vishalakshi and Singh, 2006).

3. Role of fluctuating asymmetry in sexual selection

More than two decades have passed since Markow (1987) and Møller (1990) first suggested that FA, the morphological expression of developmental instability (DI), might play a role in sexual selection and/or communication (Uetz and Taylor, 2003). FA has been used as an empirical measure of the quality of individuals competing for reproductive success and particularly of a revealing aspect of an individual's phenotype (FA-sexual selection hypothesis; Uetz and Taylor, 2003). Field studies have shown that FA is higher in secondary sexual traits, which are often costly and extravagant in pattern, colouration and size. Also, FA is more pronounced in sexual traits than non-sexual traits (Møller and Pomiankowski, 1993; Rasmuson, 2002; Vishalakshi and Singh, 2006). But the connection between FA and sexual selection has not always been found (as reviewed in Simmons et al., 1999), and thus, the precise role of FA in the sexual selection process is often controversial (Tomkins and Simmons, 2003).

3.1 Studies in favor of FA-sexual selecion hypothesis

In *D. melanogaster*, FA in sternopleural bristle was associated with male mating success in laboratory (Markow, 1987). In *D. simulans,* mating males were larger than the single males and were characterized by reduced FA (Markow and Ricker, 1992). In *D. pseudoobscura*, the body size of mated and unmated males was similar, but the mated males exhibited less FA than the single males (Markow and Ricker, 1992). Further, in *D. buzzatii,* Santos (2001) found that FA in wing length was higher in non-mating males than in mating ones.

3.2 Studies against FA-sexual selection hypothesis

In *D. mojavensis,* mating males were larger than single males but showed a similar level of FA to that observed in single males (Markow and Ricker, 1992). In another study, Markow et al. (1996) found that the body size did not differ between mated and unmated males in either *D. simulans* or *D. pseudoobscura*. However, the mating males of *D. simulans* had significantly fewer sex comb teeth and the mating males of *D. pseudoobscura* had more sternopleural bristles than the single males, but no difference was found in the levels of FA in any bilateral traits between the mated and the unmated males in either species (Markow et al., 1996). In *D. buzzatii*, it was shown that body size was positively correlated with mating success and other fitness components (Santos et al., 1992; Norry et al., 1995), but Norry et al. (1998) found no phenotypic correlation between wing asymmetry and size, suggesting that wing asymmetry does not indicate male

Table 2 : Genetic stress and fluctuating asymmetry in *Drosophila*

Stressor	Species	Materials		Traits*	Results	References
		Focal	Control			
Inbreeding						
	D. melanogaster	Inbred lines	Their hybrids	SBN	FA increased	Robertson and Reeve (1952); Mather (1953); Beardmore (1960); Reeve (1960)
	D. melanogaster	Inbred lines	Outbred lines	SBN	No effect	Fowler and Whitlock (1994)
	D. ananassae	Inbred laboratory populations		SBN, WL, W/T ratio, SCTN, ON	No effect	Vishalakshi and Singh (2008a)
	D. melanogaster	Inbred lines	Outbred lines	Wing vein	FA increased	Carter et al. (2009)
	D. subobscura	Inbred lines		ON	FA increased	Savić et al. (2008)
	D. subobscura	Isochromosomal lines (inbred)	Ditto (outbred)	Wing shape and size	FA increased	Santos et al. (2005)
Hybridization						
Interspecific crosses						
	D. melanogaster-D. simulans	Parental species	Their hybrids	SBN, WL, Fronto-orbital, frontal chaetae	FA increased	Markow and Ricker (1991)
	D. madeirensis-D. subobscura	Parental species	Their hybrids	Wing and centroid size	FA increased	Rego et al. (2006)
	D. buzzatii-D. koepferae	Parental species	Their hybrids	WL	No effect	Carreira et al. (2008)
	D. ananassae-D. pallidosa	Parental species	Their hybrids	SBN, WL, W/T ratio, SCTN, ON	No effect	Vishalakshi and Singh (2009a)
	D. mauritiana-D. melanogaster-D. simulans	Parental species	Their hybrids	WL, WD	FA increased	Stamenković-radak et al. (2009)
Intraspecific crosses						
	D. mercatorum	Parthenogenetic strain-sexually reproducing strain		SBN, WL, WD	FA increased	Andersen et al. (2002)
Mutations						
	D. melanogaster	32 lines with same genetic background with 5 visible mutations		SBN	FA increased	Bourguet (2000)
	D. melanogaster	*Notch* (*N*) mutants		6 different bristles	FA increased	Indrasamy et al. (2000)
	D. ananassae	Different mutants (single, double and triple mutations)		SBN, WL, W/T ratio, SCTN, ON	No effect	Vishalakshi and Singh (2008b)
	D. melanogaster	Heterozygous insertional mutation		Wing size and shape	FA increased	Debat et al. (2009)
	D. melanogaster			SBN, WL	No effect	Monedero et al. (1997)
	D. simulans	*Lethal hybrid rescue* (*Lhr*)		SCTN, VTN, WL, Tibia L	FA increased	Chatterjee et al. (2007)
Inversion polymorphism						
	D. subobscura	Isochromosomal lines		Wing size and shape	FA increased	Santos et al. (2005)
Directional selection						
	D. melanogaster	Faster developmental time		SBN	No effect	Shakarad et al. (2001)
	D. melanogaster	Index-based wing character		Wing traits	No effect	Pelabon et al. (2006)
	D. ananassae	Thorax length		SBN, WL W/T ratio SCTN, ON	No effect	Vishalakshi and Singh (2009b)
	D. melanogaster	Increased expression of *radius incompletus* (*ri*) mutation		Wing traits	FA increased	Imasheva et al. (1991)
Molecular chaperons						
Hsp90	*D. melanogaster*			Bristle and WL	No effect	Debat et al. (2006)
Hsp 83	*D. melanogaster*			Bristles	FA increased	Debat et al. (2006)

* SBN: sternopleural bristle number; WL: wing length; W/T ratio: wing to thorax ratio; SCTN: sex comb tooth number; ON: ovariole number; VTN: vaginal tooth number; Tibia L: tibia length; WD: wing width.

Table 3：Environmental stress and fluctuating asymmetry in *Drosophila*

Stressor	Species	Conditions	Traits*	Results	References
Temperature					
	D. melanogaster	Increase of temperature from 25 to 30°C	SBN	FA increased	Parsons (1962); Imasheva et al. (1997a, b)
	D. buzzatii	Increase of temperature from 25 to 30°C	SBN, AB, WL	FA increased	Imasheva et al. (1997a)
	D. ananassae	Low and high temperature (20, 25 to 30°C)	SBN, WL, W/T ratio, SCTN, ON	FA increased	Vishalakshi and Singh (2008c)
	D. melanogaster	Range of temperature (18, 28 and 18–28°C)	Compartments of wing	FA increased	Trotta et al. (2005)
	D. subobscura	Warm adapted versus cold adapted populations	Wing shape asymmetry	FA increased	Santos et al (2006)
	D. melanogaster	Extra Y chromosome	SBN	FA increased	Jokela and Portin (1991)
Nutrition					
	D. melanogaster		SBN, AB, ACN	FA increased	Imasheva et al. (1999)
	D. ananassae		SBN, WL, W/T ratio, SCTN, ON	No effect	Vishalakshi and Singh (2008d)
Larval crowding/density					
	D. ananassae		SBN, WL, W/T ratio, SCTN, ON	FA increased	Vishalakshi and Singh (2008d)
Multiple stress					
	D. melanogaster	Cold-ethanol-nutrition stress	OB, cross vein, WD, WL, SBN	FA increased for OB Other traits no effect	Woods et al. (1999)
Ectoparasite					
	D. nigrospiracula	Mites	SBN	FA increased	Polak (1993, 1997)
Parental age					
	D. melanogaster	Mother's age	SBN	No effect	Parsons (1962)
Heavy metals					
	D. melanogaster	Lead and benzene	SBN	FA increased	Graham et al. (1993b)
	D. melanogaster	Temperature and lead	SBN	No effect	Polak et al. (2004a)
Pesticide					
	D. melanogaster	Endosulphan	TL, WL, SBN, OB	No effect	Antipin and Imasheva (2001)
Chemical					
	D. melanogaster	Phenylthiourea	SBN	No effect	Parsons (1961)
	D. melanogaster	Arsenic	SBN	No effect	Polak et al. (2002)
Magentic fields					
	D. melanogaster	50 Hz of 1.5 and 80μ	SBN, WL	FA increased	Graham et al. (2000)
Host variation					
	D. pachea	Cactus host variation	Wing and leg size	No effect	Hurtado et al. (1997)
Seasonal change					
	D. melanogaster	Seasonal dynamics	TL, WL, SBN, OB	No effect	Antipin et al. (2001)
Habitat					
	D. serrata	Marginal versus central population	WL, WD, SBN, cross vein (wing)	No effect	Jenkins and Hoffmann (2000)

* AB: arista branches; ACN: abdominal chaetae number; SBN: sternopleural bristle number; WL: wing length; W/T ratio: wing to thorax ratio; SCTN: sex comb tooth number; ON: ovariole number; Tibia L: tibia length; WD: wing width; TL: thorax length; OB: orbital bristles.

quality in terms of phenotypic variation in body size. There is also evidence for the positive genetic correlation between sex comb size and FA in *D. bipectinata* (Polak et al., 2004b). However, in another study, Polak and Starmer (2005) found that males reared at 29°C produced smaller and less symmetrical sex combs than males developed at 25°C. Also, they reported that there was a negative correlation between sex comb size and FA generated by means of heterogeneous exposure to thermal stress; i.e., both sex comb size and symmetry decreased with increasing temperature within the range experienced by the species in nature (Polak and Starmer, 2005). The relationship between genotype, developmental stability and mating performance has been investigated in *D. immigrans* (Polak and Stillabower, 2004). Among males, there was a significant positive relationship of positional fluctuating asymmetry (PFA, Polak, 1997, for definition see Table 1) with copulation latency (time elapsed between introduction of females and copulation) and copulation duration, but not with copulation frequency. Moreover, high developmental stability lines exhibited significantly shorter copulation latency and duration compared with low developmental stability lines. When these components of sexual performance were again contrasted between lines with among-individual differences in bristle asymmetry controlled statistically, significant line effects on copulation latency and duration disappeared (Polak and Stillabower, 2004). These results suggest that deficits in the developmental apparatus underlying one particular trait can compromise individual sexual performance, and thereby weakens the hypothesis that FA is a cue of overall 'genetic quality' (Polak and Stillabower, 2004). By using comprehensive multivariate analyses, Polak and Taylor (2007) have nicely shown that fluctuating asymmetry and minor morphological abnormalities (MMAs) in a condition-dependent sexual ornament (sex comb) in *D. bipectinata* are the significant targets of selection favouring their reduced expression in a natural population of New Caledonia *D. bipectinata*. Ornament size and developmental instability (DI measured as FA and MMAs) are positively correlated genetically and phenotypically, which is in contrast to the theoretical expectation of negative size-FA scaling under the assumption that FA reveals overall genetic quality. Furthermore, they have shown that there exists a significant additive genetic variance for MMAs, demonstrating their evolutionary potential. Ornament DI in New Caledonian *D. bipectinata* is markedly elevated in comparison to the populations where such selection has not been detected, suggesting that the increased population-level DI is capacitating adaptive evolution (Polak and Taylor, 2007). In *D. ananassae*, Vishalakshi and Singh (2008e) found that the traits related to body size (thorax and wing length) of mated flies were larger than unmated ones, suggesting that sexual selection acts directly or indirectly on the body size. However, the levels of FA were similar in the mated and unmated flies for different morphological traits in both males and females, suggesting that FA cannot be used as an ideal phenotypic cue for choosy females and thereby weakens the FA-sexual selection hypothesis (Vishalakshi and Singh, 2008e).

3.3 Status of FA-sexual selection hypothesis

The inconsistency of results in the studies investigating the role of FA in sexual selection casts doubts on the existence of FA-sexual selection hypothesis. On the one side, there is evidence for the role that FA plays in sexual selection, e.g., in humans, insects, fishes and birds (for references see Polak and Taylor, 2007), but, on the other side, there are studies showing that FA cannot be considered as a reliable indicator of genetic quality (see section 3.2 for examples). So, the question arises why FA predicts sexual success in some species but not in others. The explanation for this may be that most species and populations do not have sufficient DI variation to sustain FA-mediated sexual selection (Polak and Taylor, 2007) due to the robust buffering mechanisms (Gibson and Wagner, 2000) that are maintained by canalizing selection (Polak and Taylor, 2007). As a result, the effectiveness of FA minimizes to reveal DI differences among individuals of a population, as FA is weakly correlated to the underlying DI (Houle, 2000; Polak and Taylor, 2007). Therefore, FA-sexual selection hypothesis may exist in populations with DI-enriched FA in a particular trait or in the whole genome due to genetic or environmental destabilizing factors (for references see Polak and Taylor, 2007). Otherwise, whatever FA we are getting reflects the compromised trait specific developmental buffering rather than overall genetic quality (as discussed by Polak and Taylor, 2007). But this explanation is based on the results of few studies, and therefore, in order to get in-depth knowledge of what role FA plays in sexual

selection, more investigations have to be carried out, which will definitely unveil the status of FA-sexual selection hypothesis, in general.

4. Does fluctuating asymmetry have any genetic basis?

Whether FA has any genetic basis or not is a subject for intense debate. FA is considered to be purely environmental in origin but it may also be under genetic control (Leamy 1997; Fuller and Houle, 2003). Empirical studies have shown that the heritability of FA is quite low (Mather, 1953; Thoday, 1958; Reeve, 1960), but significant heritability has also been found in some cases (Scheiner et al., 1991; Møller and Thornhill, 1997). Studies using QTL mapping and effect of single genes on FA have been used to disentangle the presence of dominance (intra-locus) and epistasis (inter-locus) interactions in the non-additive genetic architecture of FA in mice (Leamy and Klingenberg, 2005; Van Dongen, 2006).

5. Conclusions

Fluctuating asymmetry has been studied as an indicator of stress and individual quality in a wide variety of organisms ranging from viruses, animals to plants and also in fossil materials (see Graham et al., 2010, for references). FA is often considered as trait-, sex- and taxon-specific (Swaddle, 2003). However, due to inconsistency of results, we are unable to generalise any clear-cut relationships between FA and stress or individual quality, which compels us to think that FA is not a ubiquitous measure of individual quality. FA has considerable potential to assess stress and individual quality, provided the studies dealing with FA pay more attention to an appropriate methodology including sufficient statistical power and appropriate analysis (Knierim et al., 2007). This is also echoed from the statement "when studied with care, subtle deviations from symmetry (FA) offer a unique measure of developmental precision for many organisms and traits and may even yield insights into the evolution of conspicuous asymmetries" (Palmer, 1996).

Hence, it is essential to reconsider the association between FA and fitness and also the genetic architecture of FA under different stress conditions, which will help us in understanding the role of FA in macro- and micro-evolutionary processes.

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Courtship of *Drosophila*, with a special interest in courtship songs

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This paper reviews the courtship behaviour of *Drosophila*, focusing on courtship songs and types and patterns of sounds in a variety of species with regard to phylogeny. Courtship song is one of the most important signals from males to females during courtship. In most species, males emit one or two types of courtship song, such as a pulse song and a sine song. Some species produce two types of pulse songs, some a pulse song and a sine song, and others a pulse song but no sine song. In the *montium* species subgroup of the *Drosophila* (*Sophophora*) *melanogaster* species group, copulatory courtship songs are reported; some species produce before and after mounting but others only after mounting. In the *Drosophila* (*Drosophila*) *virilis* species group and *Zaprionus*, both sexes produce songs. Breeding sites, sex pheromones and other courtship signals are also examined. Mate recognition in relation to the role of courtship elements are discussed from an evolutionary point of view.

1. Introduction

Courtship behaviour in *Drosophila* was first reported by Sturtevant (1915), who used *Drosophila* (*Sophophora*) *melanogaster* (*D. ampelophila*) and described several courtship elements, including vibration, scissors movement, circling and licking. Wing vibration was pointed out as "the most noticeable act" in the courtship described in that report. A sound is produced by the vibration, which was not known at the time, but now the importance of courtship songs is widely acknowledged.

More than 4,000 species are described in Drosophilidae and more than 1,000 belong to a single genus, *Drosophila*. Although there are differences in quality (behavioural elements) and quantity of courtship behaviour between species, an outline of the courtship behaviour of a particular species is helpful for understanding the function and diversity of courtship of *Drosophila*. Here, like Sturtevant, we are concerned with *D. melanogaster*.

Courtship begins with the orientation of the male. He slows down his movements and points his head toward a female at a distance of one to two body lengths. He approaches her and when she moves, follows her. Vision is one of the most important modalities in orientation and following. In *D. melanogaster*, the frequency of and time spent on orientation decline in the dark (Willmund and Ewing, 1982; Sakai et al., 1997). When the male moves close to the female, he touches her with his foreleg (tapping). There are taste-type chemosensory bristles on the fore tarsi. He senses cuticular hydrocarbons on her body by tapping. After several rounds of tapping, he vibrates his wing at 90 degrees and produces a courtship song (Fig. 1A). The vibrating wing is, in most cases, the one closest to the female. Although it is less frequently observed, the male vibrates both wings as scissors (scissoring, Fig. 1B). Then he licks her genitalia after and/or with vibration. If the female is receptive, she moves slowly, stops walking, and spreads her vaginal plates. The male curls his abdomen and tries to grasp her abdomen with his forelegs and middle legs to copulate (a copulation attempt). If she does not reject him, genitalic coupling is achieved. If the female rejects him, she escapes from him (decamping), kicks him with her hindlegs (kicking), or moves both her wings (fluttering).

In real observations, the sequence of courtship is

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not always the linear process mentioned above. In some cases, a male repeats several behavioural elements back and forth. In other cases, especially when the male is sexually excited, some elements are omitted. If the female escapes from the male, he ceases courting.

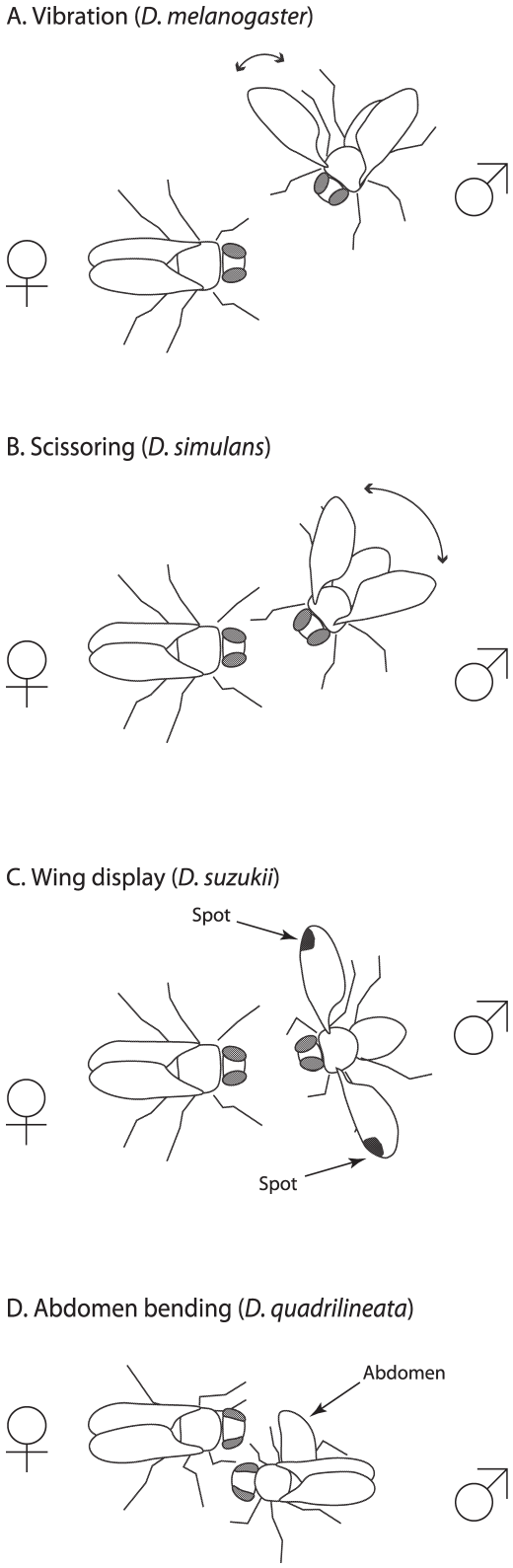

Figure 1：Wings used in courtship. Vibration in *Drosophila melanogaster* (A), scissoring in *D. simulans* (B), wing display in *D. suzukii* (C) and abdomen bending in *D. quadriliniata* (D).

Courtship elements are species specific. Some species have elements that are different from *D. melanogaster*. Others do not have some of the elements observed in *D. melanogaster*. Quantitative differences between strains or species also exist. Therefore, in order to compare courtship between species in detail, the observer may sequentially record when all the courtship elements begin and end. Data can be summarized as an ethogram or a diagram that shows transition probabilities from one element to others and also can be used for several statistical analyses.

Although vibration is the most remarkable behaviour in *Drosophila* courtship as Sturtevant (1915) described, it was about a half century before Shorey (1962) reported sounds produced by vibration (Fig. 2). Since then, courtship songs of many species have been described in *Drosophila*. In general, courtship songs are species specific, especially between sympatric species, playing an important role in the courtship of *Drosophila* and affecting the female's decision of whether or not to accept the courting male.

This review summarizes the courtship behaviour of *Drosophila* and provides an up-to-date catalog of *Drosophila* songs. Since phylogenetic constraints are one of the most important factors in the evolution of behaviour, we will discuss the behaviour in relation to the phylogeny of species of *Drosophila*. Hawaiian picture-winged *Drosophila* exhibit diversified biological traits including courtship behaviour (Spieth, 1984). The diversity and evolution of the Hawaiian species are quite interesting, but we deal with these species only as a special topic.

2. Breeding sites

A wide variety of breeding sites are used by *Drosophila*, including plants, fungi, fruits, fermenting sap (slime fluxes), cacti, flowers and crabs (Spieth, 1951; Carson, 1971; Powell, 1997). Although many species can be collected on rotting fruits and by banana bait traps, there may exist host specificity or preference. Some species use secondary host plants and host switching may have played an important role in the separation of lineages (Magnacca et al., 2008). In *D. melanogaster*, courtship and mating usually take place at feeding locations, rotting fruits, where females oviposit. Both sexes are attracted to the

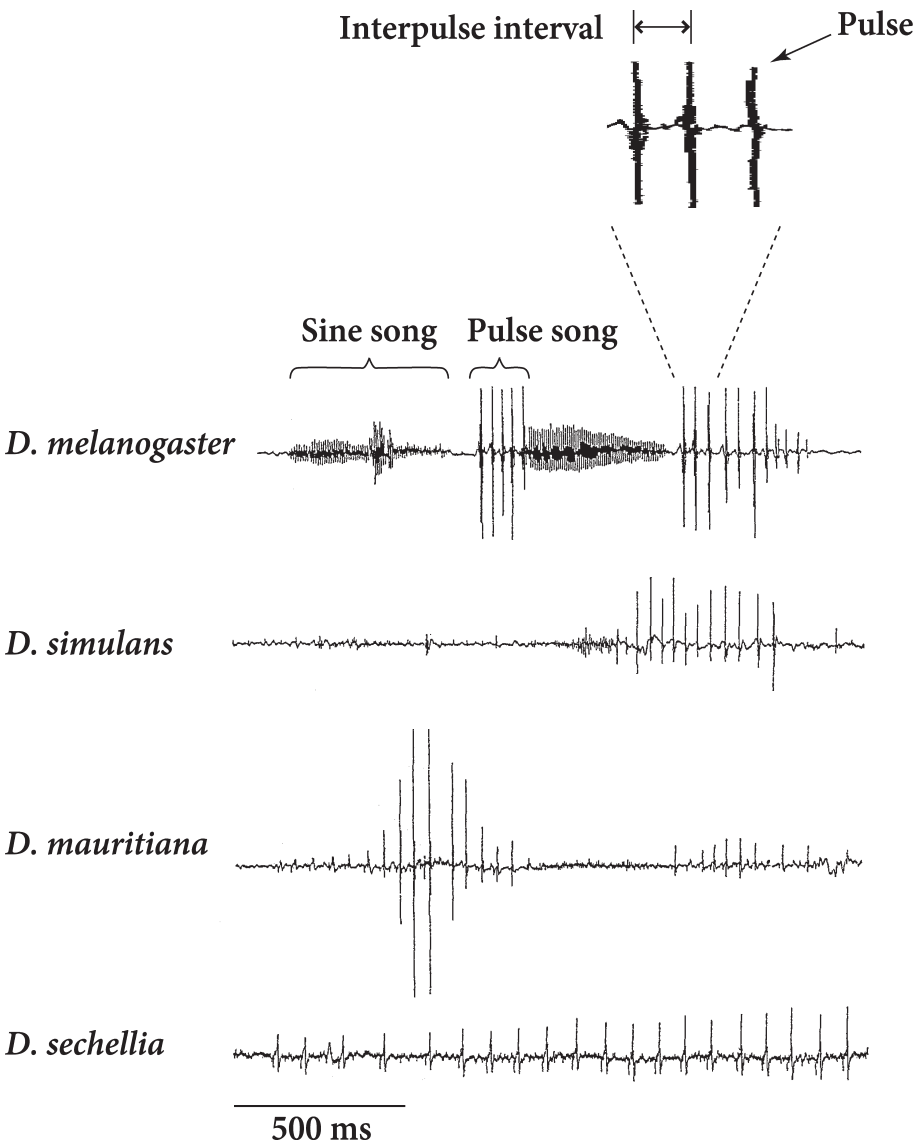

Figure 2 : Oscillogram of courtship songs in *Drosophila melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia*. Both pulse and sine songs are observed in *D. melanogaster*, *D. simulans* and *D. mauritiana*, but only pulse song in *D. sechellia*. Interpulse interval is a time interval between pulses. The original figure is from Tomaru and Oguma (2003) and modified for this review.

odours. Some species have a specific host, for example, *Gecarcinus ruricola* (black or mountain crab) for crab fly, *D. carcinophila* of the *mercatorum* species subgroup of the *D.* (*D.*) *repleta* species group and *D. endobranchia* of the *D.* (*D.*) *canalinea* species group (Carson, 1971); *Morinda citrifolia* for *D. sechellia* of the *D.* (*S.*) *melanogaster* species subgroup (Lachaise et al., 1988; R'Kha et al., 1991; Higa and Fuyama, 1993; Jones, 1998); cacti for cactophilic species of the *repleta* group (Krebs and Bean, 1991; Powell, 1997); and flowers for many species in a variety of groups, for example, *D. florae* of the *D.* (*D.*) *bromeliae* species group, species of the *D.* (*D.*) *flavopilosa* species group, *D.* (*Phloridosa*) *lutzii* (Carson, 1971), *D. oshimai* of the *suzukii* species subgroup of the *D.* (*S.*) *melanogaster* species group (Choo and Nakamura, 1973), and species of the *D.* (*S.*) *elegans* species subgroup of the *melanogaster* group (Sultana et al., 1999; Kimura and Hirai, 2001).

Feeding, mating and ovipositing do not always take place at the same site in a lot of species (Carson, 1971). Males of *D. elegans* hold territories on morning glory (*Ipomea*) flowers (Hirai and Kimura, 1997; Kimura and Hirai, 2001). In the early morning males disperse to newly opened flowers from withered flowers where they spent the night. Aggressive behaviour or fighting with intruding males is observed. A series of courtship behaviours is performed on flowers. The number of males on a single flower increases from morning to evening and the males can no longer defend their territories. In the evening, the flies stay and spend the night. Females

lay eggs on flowers in the daytime. Species of Hawaiian picture-winged *Drosophila* court and mate not at the feeding sites but on leks (Spieth, 1984). For example, males make leks on tree trunks, the undersurface of trees, or shrub stems. A female visits the leks and chooses a male to mate. In cactophilic *D.* (*D.*) *mojavensis*, males are distributed across the necrotic cactus arms in the morning and courtship and mating are observed there. The site of ovipositing is not the necrotic arms but different parts of the cacti (Krebs and Bean, 1991).

3. Courtship songs

3.1 Two types of courtship songs

Males of most species in the genus *Drosophila* emit sounds (courtship songs) toward females by vibrating their wing(s) during courtship rituals (Table 1, where the species are arranged according to their phylogenetic relationships shown in Fig. 3). Some species produce sounds by one wing, some by both wings and others use one or both wings in a species specific manner. Furthermore, males of some species vibrate wings differently for each song repertoire even in a species (Asada et al., 1992; Demetriades et al., 1999). Courtship songs of *Drosophila* can be classified into two types based on the waveforms of the sounds. The major type, "pulse song", is observed in most species producing songs. The other type is "sine song" (or "humming sound"). Both types are common in species of the genus *Drosophila*, although different names have been sometimes used to describe similar songs of different species by different authors.

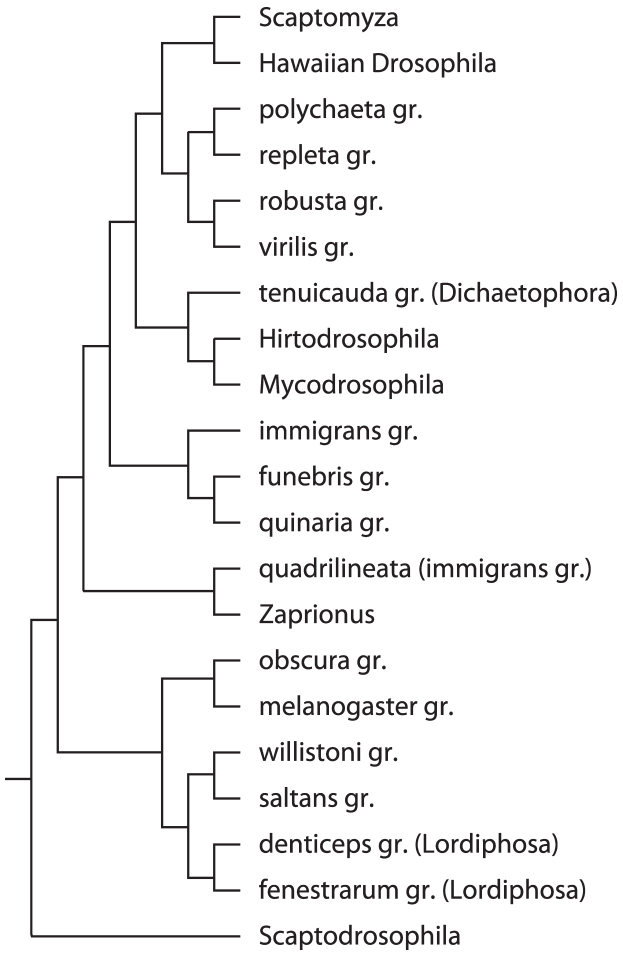

Figure 3 : Phylogenetic relationships of *Drosophila*. The diagram is based on the consensus tree of Katoh et al. (2000) and has been modified for the *Drosophila immigrans* species group (Katoh et al., 2007). Genera or species groups not listed in Table 1 are also presented.

A pulse song consists of a series of separate pulses (Fig. 2); although sometimes the pulses are connected without intervals. Each pulse is composed of several cycles of waves. A series of pulses is called a "burst" or "train". Pulse songs are characterized by several parameters such as cycle length, cycle number per pulse, pulse length, intrapulse frequency, interpulse interval, pulse number per burst, intraburst frequency and interburst interval. Different terms may be used to describe song parameters by different authors and the same terms are sometimes defined differently. Thus, one should be careful when comparing songs between species, since different terms sometimes refer to the same parameter. Sine songs are sounds without pulse structure (Fig. 2); the name "sine song" comes from the waveform which is similar to a sine wave. There are many species in which males do not produce this type of song (Table 1). Sine songs are characterized by the frequency and length of the song.

3.2 Song repertoires

Concerning song repertoires, some species have only one song and others have two or more song types. In the former case, the songs are usually pulse songs. In the latter case, combinations include two types of pulse songs, a pulse song and a sine song, or two types of pulse songs and a sine song. Thus, some species have several types of pulse songs. To date, a species with three song types (two pulse plus one sine) is *D. siamana* of the *hypocausta* species subgroup, the *D.* (*D.*) *immigrans* species group (Asada et al., 1992).

Several different terms for more than two pulse songs have been used and there are also differences how to classify the pulse songs. Two species in the *D.* (*S.*) *pseudoobscura* species subgroup (*D. pseudo-*

obscura and *D. persimilis*) have two pulse songs, a high rate repetition song (HRR) and a low rate repetition song (LRR), which differ in rates of repetition in a burst (Ewing, 1969). Similarly, five species in the *D.* (*S.*) *affinis* species subgroup (*D. affinis*, *D. algonquin*, *D. athabasca*, *D. azteca* and *D. tolteca*) have two pulse songs, high and low pulse repetition rate sounds (Chang and Miller, 1978). Species in these two species subgroups (the *pseudoobscura* and the *affinis* subgroups) have similar two type songs and both subgroups belong to the same species group, the *D.* (*S.*) *obscura* species group. Two species in the *D.* (*D.*) *funebris* species group (*D. multispina* and *D. macrospina*) have two pulse songs, primary and secondary songs, the latter being characterized by polycyclic pulses and long interpulse interval (Ewing, 1979). Twelve species in the *repleta* group (*D. anceps*, *D. meridiana*, *D. navojoa*, *D. peninsularis*, *D. stalkeri*, *D. mercatorum*, *D. canapalpa*, *D. limensis*, *D. melanopalpa*, *D. hydei*, *D. eohydei* and *D. neohydei*) have two pulse songs, A and B songs; A songs consist of simple, short pulse trains produced towards the start of courtship, while B songs tend to be more complex and occur later (Ewing and Miyan, 1986). Four species in the *bipectinata* species complex of the *D.* (*S.*) *ananassae* species subgroup of the *melanogaster* group (*D. bipectinata*, *D. parabipectinata*, *D. pseudoananassae* and *D. malerkotliana*) have two pulse songs, short and long songs (Crossley, 1986).

In the *melanogaster* subgroup, six species have both pulse and sine songs: *D. melanogaster*, *D. simulans*, *D. mauritiana*, *D. erecta*, *D. teissieri* and *D. orena* (Ewing and Bennet-Clark, 1968; Cowling and Burnet, 1981; Cobb et al., 1989), whereas *D. sechellia*, *D. yakuba* and *D. santomea* have no sine songs (Cobb et al., 1989; Watson et al., 2007; Blyth et al., 2008). Although *D. yakuba* and *D. santomea* have no sine song, they have two types of pulse songs (Demetriades et al., 1999; Watson et al., 2007; Blyth et al. 2008): primary and secondary songs that differ in interpulse interval and intrapulse frequency (Blyth et al. 2008). Even though these two species are close relatives in this species subgroup (Llopart et al., 2002), song parameters are clearly different. Primary and secondary songs were first named as clack and thud songs, respectively, by Demetriades et al. (1999), but there is a confusion surrounding the terminology of song types (Blyth et al., 2008). Therefore, here we use new terminology, primary and secondary songs, which are reclassified by Blyth et al. (2008). Two closely related species, *D. birchii* and *D. serrata* of the *D.* (*S.*) *montium* species subgroup of the *melanogaster* group (Hoikkala and Crossley, 2000), three species of the *lini* clade in the *D.* (*S.*) *kikkawai* species complex of the *montium* subgroup (*D. lini*, *D. ohnishii* and *D. ogumai*) (Wen et al., 2011), two species in the *D.* (*D.*) *virilis* species group (*D. littoralis* and *D. flavomontana*) (Hoikkala, 1985; Suvanto et al., 1994) and one species, *Hirtodrosophila mycetophaga* (Crossley, 1989) also have both pulse and sine songs. The order of pulse songs and sine songs in courtship is also species specific; species in the *melanogaster* species subgroup usually produce sine songs before pulse songs. Songs of *H. mycetophaga* look like those of *D. melanogaster*, that is, sine song followed by pulse song. On the other hand, three species of the *lini* clade produce pulse songs followed by sine song. A male of *D. littoralis* usually produces pulse songs but he produces a humming sound while circling around a female when the female does not spread her wings (acceptance posture).

Courtship songs are, in general, species specific. As mentioned in the previous section, song repertoires and structures are similar among species in the same species subgroup or species complex; in this sense, courtship songs reflect phylogenetic relationships. But parameters, such as interpulse interval and intrapulse frequency, are diverged even between closely related species. Since species of *Drosophila* producing courtship songs have at least one pulse song and a part of the species have sine song, pulse song in the genus *Drosophila* seems to be a single origin, whereas sine song is likely to have multiple origins because species producing sine song belong to different groups (Crossley, 1990). Pulse songs are often critical for species recognition for females, and therefore, it is difficult to lose. In contrast, sine songs are not species identifiers in most cases, suggesting that they are easily lost. The sound characteristics and wing movements of sine songs are similar to those of when flying (von Schilcher, 1976b; Crossley, 1990). Therefore, sine songs are easier to generate than pulse songs. An exception is the *lini* clade, where sine song seems to be more important than pulse song (Wen et al., 2011).

3.3 Role of courtship songs

Courtship songs have been suggested to: (1) lower female locomotor activity and increase female recep-

Table 1：Phylogenetic relationships and courtship song characteristics in *Drosophila*

Genus	Subgenus	Species group	Species subgroup/ phylad	Species complex	Species	Description	Pulse song		Sine song		Duet/ female song	Copulatory song	References
							IPI (ms)	IPF	Description	Frequency			
Drosophila	*Idiomyia*	picture wing (Hawaiian)	*pilimana* subgroup		*D. glabriapex*	Pulse-structured high frequency clicking sounds.	16.4 (20–21°C)	10000–14500 cps (20–21°C)					Hoikkala et al. (1989)
					D. lineosetae	Pulse-structured high frequency clicking sounds.	90.0 (20–21°C)	8300–12500 cps (20–21°C)					Hoikkala et al. (1989)
					D. fasciculisetae	Pulse-structured high frequency clicking sounds.	9.2 (20–21°C)	frequency range: 500 to >10000 Hz, dominant frequency: 5500–6000 Hz, 4600–5600 cps (20–21°C)					Hoy et al. (1988), Hoikkala et al. (1989)
			grimshawi subgroup		*D. affinidisjuncta*	Pulse-structured high frequency clicking sounds.	5.8 (20–21°C)	10100–14100 cps (20–21°C)					Hoikkala et al. (1989)
					D. disjuncta	Pulse-structured high frequency clicking sounds.	7.3 (20–21°C)	5200–6600 cps (20–21°C)					Hoikkala et al. (1989)
			vesciseta subgroup		*D. digressa*	Pulse-structured high frequency clicking sounds.	5.6 (20–21°C)	5600–12800 cps (20–21°C)					Hoikkala et al. (1989)
			planitibia subgroup		*D. cyrtoloma*	Pulse-structured song and trill (long series of stereotyped sound cycles).	165.64	85.08 Hz					Hoy et al. (1988)
					D. silvestris	Pulse song (generated by abdominal vibrations) and tone song.	45.0–65.9 (20±1°C)	175 Hz	Tone song	169.5 Hz, 90–222 Hz (20±1°C)	Duet		Hoy et al. (1988), Hoikkala and Welbergen (1995)
					D. planitibia	Pulse and humming sounds.	55.2–81.9 (20±1°C)	400–500 Hz (18–19°C)	Humming sound	109–260 Hz (20±1°C), 120–300 Hz (18–19°C)			Hoikkala and Welbergen (1995), Hoikkala and Kaneshiro (1997)
					D. heteroneura		54.5–76.2 (20±1°C)			203–250 Hz (20±1°C)			Hoikkala and Welbergen (1995)
	Drosophila	*repleta* group	*hydei* subgroup	*hydei* complex	*D. hydei*	Two pulse songs (A and B song).	A: 13.6, B: 13.6 (25.5°C)						Ewing (1979), Ewing and Miyan (1986)
					D. eohydei	Two pulse songs (A and B song).	A: 12.6, B: 12.6 (25.5°C)						Ewing (1979), Ewing and Miyan (1986)
					D. neohydei	Two pulse songs (A and B song).	A: 11.1, B: 11.1 (25.5°C)						Ewing (1979), Ewing and Miyan (1986)

Drosophila	*Drosophila*	*repleta* group	*mercatorum* subgroup		*D. mercatorum*	Two pulse songs (A and B song).	A: 8.2–9.4, B: 52.7–59.5 (25°C), A: 8.6, B: 8.6 (25.5°C)					Ewing (1979), Ikeda et al. (1980), Ewing and Miyan (1986)
					D. paranaensis	Complex pulse trains.	6.9, 14.4, 19.2 (25.5°C)					Ewing (1979), Ewing and Miyan (1986)
					D. peninsularis	Two pulse songs (A and B song).	A: 14.7, B: 6.8 (25.5°C)					Ewing (1979), Ewing and Miyan (1986)
			fasciola subgroup	*moju* complex	*D. moju*	Simple pulse song.	50.859 (25±1°C)	412.501 Hz (25±1°C)				Costa and Sene (2002)
					D. coroica	Simple pulse song.	99.050–109.101 (25±1°C)	305.459–314.364 Hz (25±1°C)				Costa and Sene (2002)
					D. ellisoni	Short pulses followed by a trill.	97.473 (25±1°C)	251.358 Hz (25±1°C)				Costa and Sene (2002)
					D. fascioloides	Short pulses followed by a trill.	125.757 (25±1°C)	362.901 Hz (25±1°C)				Costa and Sene (2002)
					D. onca	Short pulses followed by a trill.	508.091 (25±1°C)	289.26 Hz (25±1°C)				Costa and Sene (2002)
					D. rosinae	Simple but duplicated pulses.	72.34 (25±1°C)	306.624 Hz (25±1°C)				Costa and Sene (2002)
			mulleri subgroup	*eremophila* complex	*D. mettleri*	Pulse song.	35 (24±1°C)					Alonso-Pimental et al. (1995)
					D. eremophila	Pulse song.	32 (24±1°C)					Alonso-Pimental et al. (1995)
					D. micromettleri	Pulse song.	24 (24±1°C)					Alonso-Pimental et al. (1995)
				anceps complex	*D. nigrospiracula*	Pulse song.	<10 (24±1°C)					Alonso-Pimental et al. (1995)
					D. anceps	Two pulse songs (A and B song).	<10 (24±1°C), A: 9.6, B: 9.6, 23.6, 35.5 (25.5°C)					Ewing (1979), Ewing and Miyan (1986), Alonso-Pimental et al. (1995)
					D. leonis	Pulse song.	<10 (24±1°C)					Alonso-Pimental et al. (1995)
				mulleri complex	*D. mulleri*	Simple pulse trains.	25.9 (25.5°C)					Ewing and Miyan (1986)
					D. aldrichi	Simple pulse trains.	15.8 (25.5°C)					Ewing and Miyan (1986)
				buzzatii complex	*D. buzzatii*	Complex pulse trains.	8.1, 12.4 (25.5°C)					Ewing and Miyan (1986)
				longicornis complex	*D. longicornis*	Simple pulse trains.	6.8 (25.5°C)					Ewing and Miyan (1986)
				ritae complex	*D. ritae*	No patterned song.						Ewing and Miyan (1986)
				mojavensis complex	*D. arizonensis*† (*arizonae*)	Simple pulse trains.	19.8 (25.5°C)					Ewing and Miyan (1986)

Genus	Subgenus	Species group	Species subgroup/ phylad	Species complex	Species	Description	Pulse song		Sine song		Duet/ female song	Copulatory song	References
							IPI (ms)	IPF	Description	Frequency			
Drosophila	*Drosophila*	*repleta* group	*mulleri* subgroup	*mojavensis* complex	*D. mojavensis*	Simple pulse trains.	16.8 (25.5°C)						Ewing and Miyan (1986), Etges et al. (2006)
					D. navojoa	Two pulse songs (A and B song).	A: 9.8, B: 29.7 (25.5°C)						Ewing and Miyan (1986)
				stalkeri complex	*D. stalkeri*	Two pulse songs (A and B song).	A: 8.4, B: 17.5 (25.5°C)						Ewing and Miyan (1986)
				meridiana complex	*D. meridiana*	Two pulse songs (A and B song).	A: 11.0, B: 15.7, 28.7 (25.5°C)						Ewing and Miyan (1986)
					D. meridionalis	Complex pulse trains.	169.80–200.84 (25°C)	433.21–503.91 Hz (25°C)					Costa et al. (2000)
				martensis complex	*D. martensis*	Simple pulse trains.	7.1 (25.5°C)						Ewing and Miyan (1986)
			repleta subgroup	*repleta* complex	*D. repleta*	Simple pulse trains.	11 (25.5°C)						Ewing and Miyan (1986)
					D. canapalpa (synonym of *neorepleta*)	A and B songs	A: 11.6, B: 29.9 (25.5°C)						Ewing and Miyan (1986)
					D. limensis	A and B songs	A: 12.0, B: 12.5 (25.5°C)						Ewing and Miyan (1986)
					D. melanopalpa (synonym of *releta*)	A and B songs.	A: 12.4, B: 12.4, 25.1, 37.5 (25.5°C)						Ewing and Miyan (1986)
		virilis group	*montana* phylad		*D. montana*	Pulse song.	39.9, 32 (19±1°C)	259 Hz (19±1°C)			Sometimes female song.		Donegan and Ewing (1980), Hoikkala and Lumme (1987), Hoikkala and Suvanto(1999)
					D. littoralis	Pulse song and sine song.	323 (21–22°C), 318.9	346 Hz (21–22°C), 278–345 cps (20.5–21.5°C)	The secondary songs classified as sine song.				Lakovaara and Hoikkala (1979), Donegan and Ewing (1980), Hoikkala (1985), Hoikkala and Lumme (1987)
					D. flavomontana	Pulse song and sine song.	99.0		The secondary songs classified as sine song.		Sometimes female song.		Donegan and Ewing (1980), Hoikkala and Lumme (1987), Suvanto et al. (1994)
					D. lacicola	Pulse song.	81.4						Donegan and Ewing (1980), Hoikkala and Lumme (1987)

Drosophila	*Drosophila*	*virilis* group	*montana* phylad		*D. kanekoi*	Pulse song.	24.3 (21–22°C), 29.3	410 Hz (21–22°C)					Lakovaara and Hoikkala (1979), Hoikkala and Lumme (1987)
					D. borealis	Pulse song.	17.5				Duet.		Donegan and Ewing (1980), Hoikkala and Lumme (1987)
					D. ezoana	Pulse song.	43.3 (21–22°C)	455 Hz (21–22°C)			Sometimes female song.		Lakovaara and Hoikkala (1979), Donegan and Ewing (1980)
			virilis phylad		*D. virilis*	Pulse song.	20.5 (21–22°C), 18.3–22.2 (21±1°C), 20.9–21.4	306 Hz (21–22°C), 240–300 cps (21±1°C)			Sometimes female song.		Lakovaara and Hoikkala (1979), Donegan and Ewing (1980), Hoikkala and Lumme (1984), Hoikkala and Lumme (1987)
					D. lummei	Pulse song.	19.5 (21–22°C), 17.1–18.6 (21±1°C), 18.8	293 Hz (21–22°C), 275–285 cps (21±1°C)					Lakovaara and Hoikkala (1979), Hoikkala and Lumme (1984), Hoikkala and Lumme (1987)
					D. a. americana	Pulse song.	19.3				Duet.		Donegan and Ewing (1980), Hoikkala and Lumme (1987)
					D. a. texana	Pulse song.	19.8						Hoikkala and Lumme (1987)
					D. novamexicana	Pulse song.	20						Hoikkala and Lumme (1987)
Hirtodrosophila					*H. mycetophaga*	Pulse song and sine song.	46			82–180 Hz			Crossley (1989)
Drosophila	*Drosophila*	*immigrans* group	*hypocausta* subgroup		*D. hypocausta*	Pulse song and sine song.	7.8 (25°C)		Started with minimum frequency, reached a maximum and then returned to the minimum.				Asada et al. (1992)
					D. neohypocausta	No wing movement.							Asada et al. (1992)
					D. siamana	Two type pulse songs (emitted by flapping-vibration and by flapping) and sine song.	flapping-vibration: 68.3, flapping: 12.3 (25°C)		irregular sine sound.				Asada et al. (1992)

Genus	Subgenus	Species group	Species subgroup/ phylad	Species complex	Species	Description	Pulse song		Sine song		Duet/ female song	Copulatory song	References
							IPI (ms)	IPF	Description	Frequency			
Zaprionus	*Drosophila*	*funebris* group	*funebris* subgroup		*D. funebris*	One type of pulse songs (primary song).	8.4 (25.5±0.5°C)						Ewing (1979)
			macrospina subgroup		*D. subfunebris*	One type of pulse songs (secondary song).	8.6 (single cycle pulses interspersed in intervals between secondary song), 138.8 (secondary song) (25.5±0.5°C)						Ewing (1979)
					D. m. macrospina	Two types of pulse songs (primary and secondary songs).	Primary song: 12.3, Secondary song: 75.0 (25.5±0.5°C)						Ewing (1979)
					D. m. limpiensis	Two types of pulse songs (primary and secondary songs).	Primary song: 10.7, Secondary song: 64.4 (25.5±0.5°C)						Ewing (1979)
					D. multispina	Two types of pulse songs (primary and secondary songs).	Primary song: 9.9, Secondary song: varied 26.7–17.1–41.6–52.6 (25.5±0.5°C)						Ewing (1979)
					D. phalerata	Only one type of song. Copulatory song.	367.55 (21±1°C)	263.19 Hz (21±1°C)				Sing during copulation and little singing before mounting.	Neems et al. (1997)
		quinaria group			*D. kuntzei*	Only one type of song.	12.71–14.33 (21±1°C)	255–270 Hz (21±1°C)					Neems et al. (1997)
					D. limbata	Only one type of song.	14.76–16.39 (21±1°C)	245–265 Hz (21±1°C)					Neems et al. (1997)
					D. transversa	Only one type of song.	29.51–40.56 (21±1°C)	292–306 Hz (21±1°C)					Neems et al. (1997)
		immigrans group*	*quadrilineata* subgroup*		*D. quadrilineata**	No wing vibration/ display.							Tomaru et al. (2006)
Zaprionus	*Zaprionus*	*armatus* group	*tuberculatus* subgroup		*Z. tuberculatus*	Type 1 (monocyclic), Type 2 (mono or polycyclic).	Type 1: 12.4 (18–20°C) Type 2: 26.6 (18–20°C) Type 1: 10.7 (24–26°C) Type 2: 28.9 (24–26°C)	Type 2: 170 Hz (24–26°C)			Two types of songs, pulse and continuous tone sound.		Bennet-Clark et al. (1980)

Zaprionus	*armatus* group	*tuberculatus* subgroup	*Z. sepsoides*	Type 1 (monocyclic), Type 2 (polycyclic).	Type 1: 11.2 (18–20°C) Type 2: 42.0 (18–20°C) Type 1: 11.1 (24–26°C) Type 2: 40.4 (24–26°C)	Type 2: 230 Hz (18–20°C) Type 2: 185 Hz (24–26°C)			Two or possibly three types of songs.	Bennet-Clark et al. (1980)
			Z. mascarensis	Monocyclic, erratic period and erratic sequence duration.	81.7 (18–20°C)				Two types of songs.	Bennet-Clark et al. (1980)
		vittiger subgroup	*Z. ornatus*	Type 1 (monocyclic), Type 2 (polycyclic).	Type 1: 20.0 (18–20°C) Type 2: 75 (18–20°C)	Type 2: 270 Hz (18–20°C)			Polycyclic pulse song.	Bennet-Clark et al. (1980)
			Z. karoleu	Type 1 (monocyclic), Type 2 (single pulse; this "pulse" seems to be sine-song like).	Type 1: 17.1 (18–20°C) Type 2: 960 (18–20°C)		Type 2: 335 Hz (18–20°C)	Polycyclic pulse song.		Bennet-Clark et al. (1980)
	inermis group		*Z. ghesquierei*	Type 1 (monocyclic), Type 2 (polycyclic).	Type 1: 20.5 (18–20°C) Type 2: 164 (18–20°C)	Type 2: 480 Hz (18–20°C)			Pulsed whining song.	Bennet-Clark et al. (1980)
Drosophila	*obscura* group	*affinis* subgroup	*D. affinis*	Two types of sounds (Low and High pulse repetition rate sounds).	28.2, 32.8 (25°C), Low pulse repetition rate sound: 23.6–28.2, High pulse repetition rate sounds: 3.6–8.9 (22–26°C)	238 Hz (25°C), Low pulse repetition rate sound: 346.8–442.6 Hz, High pulse repetition rate sounds: 440.7–890.1 Hz (22–26°C)				Ewing and Bennet-Clark (1968), Chang and Miller (1978)
			D. algonquin	Two types of sounds (Low and High pulse repetition rate sounds).	53.3 (25°C), Low pulse repetition rate sound: 25.3–33.2, High pulse repetition rate sounds: 3.3–5.3 (22–26°C)	226 Hz (25°C), Low pulse repetition rate sound: 214.3–368.4 Hz, High pulse repetition rate sounds: 531.7–833.3 Hz (22–26°C)				Ewing and Bennet-Clark (1968), Chang and Miller (1978)
			D. athabasca	Two types of sounds (Low and High pulse repetition rate sounds) (some semispecies rarely show a type of sound).	11.7 (25°C), Low pulse repetition rate sound: "eastern A" 23–26, "eastern B" 11.24, "western-northern" 11.0 (22–26°C)	444 Hz (25°C), Low pulse repetition rate sound: 400–600 Hz (22–26°C)				Ewing and Bennet-Clark (1968), Chang and Miller (1978)
			D. azteca	Two types of sounds (Low and High pulse repetition rate sounds).	High pulse repetition rate sounds: 11.4–15.5 (22–26°C)	High pulse repetition rate sounds: 450–500 Hz (22–26°C)				Chang and Miller (1978)

Genus	Subgenus	Species group	Species subgroup/ phylad	Species complex	Species	Description	Pulse song		Sine song		Duet/ female song	Copulatory song	References
							IPI (ms)	IPF	Description	Frequency			
Drosophila	*Sophophora*	*obscura* group	*affinis* subgroup		*D. tolteca*	Two types of sounds (Low and High pulse repetition rate sounds).	Low pulse repetition rate sound: 9.7, High pulse repetition rate sounds: 2.5–5.4 (22–26°C)	Low pulse repetition rate sound: 507.9–666.7 Hz, High pulse repetition rate sounds: 727.7 Hz (22–26°C)					Chang and Miller (1978)
					D. narragansett	One type of pulse song (low rate repetition sound).	97.27 (22–26°C)	437.5–509.8 Hz (22–26°C)					Chang and Miller (1978)
			obscura subgroup		*D. ambigua*	Similar to that of *D. pseudoobscura.*	171, 38.4 (25°C)	452, 294 Hz (25°C)					Ewing and Bennet-Clark (1968)
			pseudoobscura subgroup		*D. pseudoobscura*	LRR & HRR.	186, 37.9 (25°C) HRR: 42.6, LRR: 15 pulses/s (23°C)	541, 258 Hz (25°C)					Ewing and Bennet-Clark (1968), Ewing (1969)
					D. persimilis	LRR & HRR.	53.7 (25°C), HRR: 66.8, LRR: omitted or very few pulses (23°C)	528 Hz (25°C)					Ewing and Bennet-Clark (1968), Ewing (1969)
			subobscura subgroup		*D. subobscura*	Wing spread horizontally, but no vibration/ sound detected.							Ewing and Bennet-Clark (1968)
		melanogaster group	*ananassae* subgroup	*ananassae* complex	*D. ananassae*	Pulse song.	10.30–11.27 (25±1°C), 3–11 (25°C)	370.88–431.26 Hz (25±1°C), 475 cps (25°C)					Ewing and Bennet-Clark (1968), Yamada et al. (2002b)
					D. pallidosa	Pulse song.	10.25–11.22 (25±1°C)	416.37–494.60 Hz (25±1°C)					Yamada et al. (2002b)
				bipectinata complex	*D. bipectinata*	Two pulse songs (short and long songs).	8.38 (25°C), Short song: 10.1, Long song: 11.0 (22±1°C)	576 cps (25°C)					Ewing and Bennet-Clark (1968), Crossley (1986)
					D. parabipectinata	Two pulse songs (short and long songs).	Short song: 12.8, Long song: 14.1 (22±1°C)						Crossley (1986)
					D. pseudoananassae	Two pulse songs (short and long songs).	Short song: 13.6, Long song: 11.0 (22±1°C)						Crossley (1986)
					D. malerkotliana	Two pulse songs (short and long songs).	Short song: 9.2, Long song: 9.6 (22±1°C)						Crossley (1986)
			elegans subgroup		*D. elegans*	Spotted wings. Wing display.							Kopp and True (2002)
			melanogaster subgroup	*melanogaster* complex	*D. melanogaster*	Pulse and sine songs.	33.8 (25°C), 30.8 (25±0.5°C)	324 cps (25°C), 282.2 Hz (25±0.5°C)		169.9 Hz (25 ± 0.5°C)			Ewing and Bennet-Clark (1968), Cowling and Burnet (1981)

Drosophila	*Sophophora*	*melanogaster* group	*melanogaster* subgroup	*melanogaster* complex	*D. simulans*	Pulse and sine songs.	48.5 (25°C), 55.2 (25±0.5°C)	391 cps (25°C), 483.0 Hz (25±0.5°C)		196.8 Hz (25±0.5°C)			Ewing and Bennet-Clark (1968), Cowling and Burnet (1981)
					D. mauritiana	Pulse and sine songs.	45.9 (25±0.5°C)	507.3 Hz (25±0.5°C)		191.4 Hz (25±0.5°C)			Cowling and Burnet (1981)
					D. sechellia	Only pulse song, no sine song.	86.3 (25±0.5°C)					Copuratory song is reported, but sound producer is not clear.	Cobb et al. (1989)
				yakuba complex	*D. yakuba*	Two types of pulse songs (primary and secondary songs), no sine song.	primary song: 129.3, secondary song: 100.1 (25±3°C)	primary song: initial 337.1 Hz, later 436.1 Hz, secondary song: initial 134.9 Hz, later 173.3 Hz (25±3°C)					Blyth et al. (2008), (Cowling and Burnet, 1981; Demetriades et al., 1999; Watson et al., 2007)
					D. santomea	Two types of pulse songs (primary and secondary songs), no sine song.	primary song: 73.84, secondary song: 59.28 (25±3°C)	primary song: initial 375.9 Hz, later 488.1 Hz, secondary song: initial 176.6 Hz, later 206.4 Hz (25±3°C)					Blyth et al. (2008), (Watson et al., 2007)
					D. teissieri	Pulse and sine songs.	20.0 (25±0.5°C)	316.5 Hz (25±0.5°C)		105.4 Hz (25±0.5°C)			Cowling and Burnet (1981)
				erecta complex	*D. erecta*	Pulse and sine songs.	40.9 (25±0.5°C)	327.6 Hz (25±0.5°C)		262.2 Hz (25±0.5°C)			Cowling and Burnet (1981)
					D. orena	Pulse and sine songs.	40.0 (25±0.5°C)			322.6 Hz (25±0.5°C)		Copuratory song is reported, but sound producer is not clear.	Cobb et al. (1989)
			montium subgroup	*auraria* complex	*D. auraria*	Pulse song, copulatory song.	18.9–21.4 (24–25°C)	98.9–101.2 Hz (24–25°C)				before mounting and during/after mounting	Tomaru and Oguma (1994a)
					D. biauraria	Pulse song, copulatory song.	12.4–13.2 (24–25°C)	112.9–163.2 Hz (24–25°C)				before mounting and during/after mounting	Tomaru and Oguma (1994a)
					D. subauraria	Pulse song, copulatory song.	11.1–11.6 (24–25°C)	107.0–184.1 Hz (24–25°C)				before mounting and during/after mounting	Tomaru and Oguma (1994a)
					D. triauraria	Pulse song, copulatory song.	15.4–16.1 (24–25°C)	106.2–127.6 Hz (24–25°C)				before mounting and during/after mounting	Tomaru and Oguma (1994a)

Genus	Subgenus	Species group	Species subgroup/ phylad	Species complex	Species	Description	Pulse song		Sine song		Duet/ female song	Copulatory song	References
							IPI (ms)	IPF	Description	Frequency			
Drosophila	*Sophophora*	*melanogaster* group	*montium* subgroup	*auraria* complex	*D. quadraria*	Pulse song, copulatory song.	15.6 (24–25°C)	128.9 Hz (24–25°C)				before mounting and during/after mounting	Tomaru and Oguma (1994a)
				kikkawai complex	*D. lini*	Pulse and sine songs, copulatory song.	82.85 (23±1°C)			253.4 Hz (23±1°C)		Only during/after mounting	Wen et al. (2011)
					D. ohnishii	Pulse and sine songs, copulatory song.	86.75 (23±1°C)			193.0 Hz (23±1°C)		Only during/after mounting	Wen et al. (2011)
					D. ogumai	Pulse and sine songs, copulatory song.	88.91 (23±1°C)			246.7 Hz (23±1°C)		Only during/after mounting	Wen et al. (2011)
				serrata complex	*D. birchii*	Most males produced only one song that is no-pulse structured, resembled sine song more than pulse song. Some males had a clear pulse structure sounds.	14 (24–26°C)	400–450 Hz (24–26°C)		150–160 Hz (24–26°C)		Only during/after mounting	Hoikkala and Crossley (2000)
					D. serrata		28.0–35.5 (24–26°C)	400–450 Hz (24–26°C)		140 Hz (24–26°C)		Only during/after mounting	Hoikkala and Crossley (2000)
			suzukii subgroup		*D. suzukii*	Spotted wings. Wing display, but no vibration.							Kopp and True (2002)
					D. pulchrella	Spotted wings. Wing display. High amplitude, nonrhythmic “toot” sounds, 422–479 Hz.							Kopp and True (2002), Lai et al. (2009)
					D. biarmipes	Spotted wings. Wing display. High amplitude, nonrhythmic “toot” sounds, 259–352 Hz.							Kopp and True (2002), Lai et al. (2009)
			takahashii subgroup		*D. prostipennis*	Spotted wings. Wing display. Short, high frequency pulse structured song.	4	520 Hz					Kopp and True (2002), Lai et al. (2009)
		willistoni group				All species have pulse song, but some species have multiple song types.							Gleason and Ritchie (1998)

Scaptodrosophila		*coracina* group		*lativittata* complex	*S. lativittata*	Pulse song.	10.5						Crossley (1989)
					S. enigma	Pulse song.	33.3						Crossley (1989)
					S. howensis	Pulse song.	17.6						Crossley (1989)
					S. nitidithorax	Pulse song.	11.0						Crossley (1989)
					S. specensis	Pulse song.	16.3						Crossley (1989)
					S. novamaculosa	Pulse sounds are produced by abdomen movement in both male and female. Wing movement is silent.					Duet		Crossley (1989)

IPI: interpulse interval, IPF: intrapulse frequency, with recording temperature also shown when possible.

* *Drosophila quadrilineata* is in a different clade from other species of the *immigrans* group (Katoh et al., 2007). See also Fig. 3.

† A junior synonym of *Drosophila mojavensis* Patterson, in Patterson & Crow, 1940

tivity (Bennet-Clark and Ewing, 1967; von Schilcher, 1976a, b; Crossley and Bennet-Clark 1993; Crossley et al., 1995), (2) increase male activity (von Schilcher, 1976a; Crossley et al., 1995; Eberl et al., 1997), (3) act as an indicator for sexual selection, (4) provide species specific signals for mating with an appropriate partner.

Although the first role is suggested in both sine and pulse songs in *D. melanogaster* (von Schilcher, 1976a, b; Crossley et al., 1995), pulse song acts as a critical signal in species recognition. As shown by playback song experiments, species specific interpulse interval is a parameter fascinating to females in the *melanogaster* subgroup (Bennet-Clark and Ewing, 1969; von Schilcher, 1976a, b), the *D.* (*S.*) *auraria* species complex of the *montium* subgroup (Tomaru et al., 1995) and the *virilis* group (Isoherranen et al., 1999b; Saarikettu et al., 2005). These findings mean that the interpulse interval is one of the critical signals to maintain species and isolate different species. Although mate recognition is performed by both sexes, usually males act first in the courtship of *Drosophila*. Males choose a mate by assessing signals emitted from females; a well-known signal is female cuticular hydrocarbons that function as a sex pheromone. The females then decide to accept or reject male courtship by examining a male-producing signal; a well-known signal is male courtship songs.

Interpulse intervals are reported to vary within a series of songs in the species of the *melanogaster* subgroup and the *pseudoobscura* subgroup (Kyriacou and Hall, 1980; Alt et al., 1998; Noor and Aquadro, 1998; Demetriades et al., 1999). The fluctuating cycle of interpulse interval variation within an individual is thought to be important for mating in the *melanogaster* subgroup (Kyriacou and Hall, 1982; Ritchie et al., 1999), but not likely in the *pseudoobscura* subgroup (Noor and Aquadro, 1998).

Importance and effects of conspecific and heterospecific songs differ depending on the species. In some cases, heterospecific songs are more important to avoid an inappropriate mate and conspecific song is not necessarily needed for a successful mating, for example, a species pair of *D.* (*S.*) *ananassae* and *D.* (*S.*) *pallidosa* (Yamada et al., 2002a, 2008), the *auraria* complex (Tomaru et al., 1995, 1998), *D. virilis* and *D. littoralis* (Hoikkala and Aspi, 1993; Isoherranen et al., 1999a). Even in such species, conspecific acoustic signals have a role in enhancing female receptivity. In some other cases, the priority of conspecific song is very high, for example, *D.* (*D.*) *montana* (Liimatainen et al., 1992; Hoikkala and Aspi, 1993), *D.* (*D.*) *ezoana* (Hoikkala and Aspi, 1993), *D.* (*Dorsilopha*) *busckii* (Bixler et al., 1992), *D. sechellia* (Tomaru et al., 2000, 2004) and one of the strains of *D. pallidosa* (Doi et al., 2001); they do not mate without conspecific songs. In other cases, species specificity in song parameters is not critical but facilitates faster mating.

3.4 Copulating songs

Males of the majority of *Drosophila* species produce songs during courtship rituals before mounting females. Some species, however, are known to show copulatory courtship (Spieth, 1952); they produce songs during mounting (when a male is trying to get on a female) and after mounting (when he is completely on her). Most of the species showing copulatory courtship belong to the *montium* subgroup, including the species of the *auraria* complex (Tomaru and Oguma, 1994a, 1994b), of the *lini* clade in the *kikkawai* complex (Wen et al., 2011) and of the *D.* (*S.*) *serrata* species complex (Hoikkala and Crossley, 2000). Outside the *montium* subgroup, copulating songs are reported in *D.* (*S.*) *algonquin* from the *affinis* subgroup (Chang and Miller, 1978). Copulating songs were also reported in two species from the *melanogaster* subgroup, *D. sechellia* and *D. orena*, but it is not clear which sex produces the sounds (Cobb et al., 1989).

Within the species producing copulating songs, some species produce songs before and during/after mounting and the others only during/after mounting. Those which produce songs before and during/after mounting are species of the *auraria* complex (Tomaru and Oguma, 1994a, 1994b) and *D. algonquin* (Chang and Miller, 1978). In contrast, three species of the *lini* clade and two species of the *serrata* complex usually do not produce songs before mounting, but do only during/after mounting. In these species, a female receives acoustic signals only after mounting. If she judges that a mounting male is not an appropriate mate, she refuses him physically, for example, by fluttering and kicking. The situation in species of the *serrata* complex and the *lini* clade looks the same, but the significance of the songs is very different. Courtship song is unlikely to be a critical factor in the discrimination between *D. serrata* and *D. birchii*, because the females discriminate against wingless males (i.e., no wing vibrations, no songs) during copulation (Hoikkala and Crossley, 2000). On the other

hand, in the three species of the *lini* clade, male songs emitted during/after mounting seem to be critical in mate discrimination (Oguma et al., 1995; Wen et al., 2011), even though they are produced only after mounting.

3.5 Rejecting sounds by females

In contrast to males, females usually do not emit specific sounds (Ewing and Bennet-Clark, 1968). Although female wing movements such as fluttering produce sounds, they are usually not a constant acoustic signal but rather irregular sound noise, often louder than male courtship songs (our observations). These sound noises are considered a signal of rejection. Such wing fluttering is observed in many species (Ewing and Bennet-Clark, 1968; Ikeda, 1985; Crossley, 1986; Paillette et al., 1991; Satokangas et al., 1994; Suvanto et al., 1994; Oguma et al., 1995; Tomaru et al., 1998; Yamada et al., 2008). Female wing fluttering was observed especially in heterospecific pairs between species of the *lini* clade in the *kikkawai* complex (Oguma et al., 1995; Wen et al., 2011), the *auraria* complex (Tomaru et al., 1998) and the *D.* (*S.*) *ananassae* species complex (Yamada et al., 2008); furthermore, the flutterings cause male courtship termination (Yamada et al., 2008). This behaviour is observed among not only females but also males (Ikeda 1985; Paillete et al., 1991; Satokangas et al., 1994; Suvanto et al., 1994; Saarikettu et al., 2005; Yamada et al., 2008). Therefore, fluttering and its effect are likely to be universal between species and sexes in *Drosophila*, but it is not known which signals emitted by the fluttering are effective among acoustic, visual and pressure sense.

3.6 Female song: duet

Females of species in the *virilis* group (*D. americana*, *D. kanekoi*, *D. borealis*, *D. lummei*, *D. ezoana*, *D. montana*, *D. virilis*, *D. flavomontana*, *D. lacicola* and *D. littoralis*) are known to produce songs called "duet", because both males and females produce songs during courtship (Donegan and Ewing, 1980; Hoikkala, 1985). Donegan and Ewing (1980) reported that the female started singing before the male. The female song has a role to induce the males to court. Actually, the female does not repel a male after producing the songs and immediately accepts a courting male (Satokangas et al., 1994). The female song observed in the *virilis* group is not common in the genus *Drosophila*, but similar female songs are known in species of the genus *Zaprionus*, a close relative of the genus *Drosophila* (Bennet-Clark et al., 1980).

3.7 Sounds produced not by wing vibration

Insects are known to use various organs to produce sounds for communication (Ewing, 1989). *Drosophila* males produce courtship songs by wing vibrations, but there are exceptional instances where organs other than the wings are used. Hawaiian *Drosophila* species produce much higher carrier frequency sounds that range from 500 Hz to greater than 10 kHz (Hoy et al., 1988; Hoikkala et al., 1989), while the songs of most *Drosophila* are less than 500 Hz. In one of the Hawaiian *Drosophila* species, *D. fasciculisetae*, males vibrate their wings when they produce sounds, but those sounds are not produced by movements of the entire wing vane because the sounds were generated even when the wings were removed (Hoy et al., 1988). *Drosophila silvestris*, another Hawaiian *Drosophila*, produces pulse song and tone song; the pulse song is generated by low-amplitude abdominal vibration (Hoy et al., 1988).

The auditory organs of flies are considered to be aristae and Johnston's organs inside antennae. As the range of frequencies those organs can perceive is between 150 and 500 Hz (Ewing, 1978), the high frequencies observed in the songs of the Hawaiian *Drosophila* exceed the hearing ability by the organs. Hoikkala and Moro (2000) examined the body structures of flies by scanning electron microscopy and found several features on the legs and wings sensilla which could play a part in sound/vibration perception, although it is not known if these structures function to hear the sounds or not.

3.8 Genetic basis of courtship song

Courtship songs are described in many species of *Drosophila* (Table 1), but extensive analyses of behaviour, genetics and evolution have been done only in some species groups including the *melanogaster* subgroup, the *virilis* group, the *auraira* complex and the *pseudoobscura* subgroup. Mutations and the genetic basis of courtship songs were reviewed by Gleason (2005).

Here we deal with the *period* gene for an example. Fluctuation cycle of interpulse interval (IPI cycle or Kyriacou and Hall cycle [K&H cycle]) is controlled by a circadian clock gene, *period* (*per*) (Kyriacou and Hall, 1980, 1986; Demetriades et al., 1999; Ritchie et al., 1999). The IPI cycle is shorter in per^s, an allele that shortens circadian periods, longer

in *per*^{L}, an allele that prolongs circadian periods, and arrhythmic in *per*^{0}, an allele of arrhythmic circadian periods. It should be noted that the *period* gene does not affect the average value of interpulse interval, indicating that interpulse interval is not determined by the *period* gene.

4. Hearing of songs

Many insects produce sounds in various ways to communicate between individuals, sexes and species. Mechanisms of sound generation are classified into several types: percussion, air expulsion, vibration, tymbal mechanisms and stridulation (Ewing, 1989). A mechanism most *Drosophila* species use is the vibration of their wings. This is typical not only in the genus *Drosophila*, but also in the order Diptera (Ewing, 1989). As the characteristics of a sound depend on the organ generating it, hearing ability and character depend on the hearing organ. Major hearing organs of insects are tympanal and flagellar organs. Tympanal organs are sound pressure detectors, which are good at detecting long distance sounds and wide range of frequencies such as 3 to 150 kHz (Ewing, 1989). On the other hand, flagellar organs are particle velocity detectors suitable for detecting near field sounds (Bennet-Clark, 1971; Eberl, 1999). Crickets, one of the insects that have the tympanal organs, sing a call song that is loud and high in frequency (more than 10 kHz) from a long distance, but flies usually do not produce such a call song. Flies in the genus *Drosophila* sing small sound courtship songs that are emitted from near field distance as they can touch and that we cannot hear without amplification.

The auditory organs in *Drosophila* are the flagellar type organs consisted from an arista that is the 6th branch of antennae and Johnston's organ located inside the second segment of antennae (Todi et al., 2004). The courtship songs are low frequency sounds of less than 500 Hz. Furthermore, females whose aristae were surgically removed showed reduced sexual receptivity in conspecific crosses (Manning, 1967; Tomaru et al., 1998; Tomaru et al., 2000; Doi et al., 2001; Yamada et al., 2008). These results indicate that the aristae and Johnston's organs are the receptors for courtship songs. Direct electrophysiological experiments using *D. funebris* showed that the antennae (including the aristae and Johnston's organ) of *Drosophila* can detect acoustic signals such as sine and pulse songs; then the organs could detect species differences in interpulse intervals (Ewing, 1978). Eberl et al. (2000) examined electrophysiological responses of the antennae using *D. melanogaster*. They can respond to simulated sine songs of 160 and 250 Hz, but their response to a 500 Hz sine song was very poor. Sine song frequencies of the species in the *melanogaster* subgroup are 100–350 Hz (Cowling and Burnet, 1981; Cobb et al., 1989). Frequency responses are well tuned by physical structures of antennae, neural inputs of sound signals and projection to the center, and highly adapted for songs (Göpfert and Robert, 2001, 2002; Kamikouchi et al., 2009; Yorozu et al., 2009). From these lines of evidence, the aristae and Johnston's organs are well tuned to detect courtship sounds, or it can be said that males produce songs tuned to female hearing ability.

5. Courtship without song

Although vibration is most noticeable in the courtship of *D. melanogaster* (Sturtevant, 1915), there are a number of species in which the males do not vibrate to produce sounds (Table 1). In species without songs, it is very likely that there is a courtship signal from males to females. In some species, males have a remarkable black spot on the tip of each wing. The spots seem to be an important visual signal in courtship. Species with spotted wings are found in the *elegans*, the *rhopaloa*, the *suzukii* and the *takahashii* species subgroups of the *melanogaster* group (Kopp and True, 2002; Yeh et al., 2006). However, not all male members of these subgroups have spotted wings. During courtship, the male of a species with spotted wings spread his wings in front of the courting female as if to display the spots (Fig. 1C). In contrast, this display behaviour is less frequently found, or absent, in related species without spots. This behavioural difference suggests that the wing spots are an important visual signal during courtship. One such species is *D. suzukii*, a pest of plants, especially cherries, berries and other skinny fruits (Matsumura, 1931; Baufeld et al., 2010; Walsh et al., 2010). Males whose wing spots were surgically removed showed reduced copulation frequency compared with intact males, but there was no significant difference in copulation frequency when the experiments were done in the dark (Fuyama, 1979). Males of *D. pulchrella*, a spotted wing species of the *suzukii* subgroup, produced high

amplitude, nonrhythmic toot sounds (Lai et al., 2009), suggesting that not only a visual signal but also sounds may play a role in the courtship of this species.

In *D.* (*D.*) *quadrilineata*, the male bends his abdomen and points its tip towards a courting female (Tomaru et al., 2006). The male never vibrates or displays his wings during courtship but frequently bends his abdomen (Fig. 1D). Although the abdomen bending can be a visual signal from a male to a female, the male may discharge volatile sex pheromones while bending his abdomen. A culture vial of this species has a characteristic odour and human observers can distinguish between a vial that has been housed with males and that with females. Behavioural assays will clarify whether or not volatile male sex pheromones are involved in the courtship of *D. quadrilineata*.

In Hawaiian *Drosophila*, males' liquid pheromones seem to play a role in courtship. Males of some species excrete a liquid from their anus onto the lek substrate, and in other species males drag the tip of their abdomen to advertise their presence to females (Spieth, 1984). Again the odour can be detected by the human nose. In addition, males of some species curl the abdomen up and forward and direct its tip towards the females' face during their courtship. The droplet is, therefore, released very close to the antennae of the females. In other species, males release a liquid during courtship and vibrate their wing as if fanning the odour to the females.

6. Sex pheromone

Cuticular hydrocarbons cover the insect body (Blomquist, 2010). They are long chain molecules and less volatile, and protect insects from desiccation and water. The components of cuticular hydrocarbons are classified into n-alkenes, methyl-branched components and unsaturated hydrocarbons. The role of cuticular hydrocarbons, their biosynthesis, their production and their detection have been best studied in *Drosophila* (Ferveur and Cobb, 2010). The contents of cuticular hydrocarbons differ between species and/or sexes. In *Drosophila*, species specific and/or sex specific unsaturated cuticular hydrocarbons play a role in contact sex pheromones. Ferveur and Cobb (2010) recently reviewed the behavioural and evolutionary roles of cuticular hydrocarbons, and Wicker-Thomas and Chertemps (2010) did the genetics and biosynthesis.

A female sex pheromone of *Drosophila* was first identified in *D. melanogaster* (Antony et al., 1985). It is (*Z,Z*)-7,11-heptacosadiene, a long chain diene ($C_{27:2}$) and a major component of cuticular hydrocarbons in *D. melanogaster* females, and acts as a sex pheromone. Although some species have sexually monomorphic cuticular hydrocarbons, *D. melanogaster* is a sexually dimorphic species. A major component of males of *D. melanogaster* is (*Z*)-7-tricosene (C_{23}). Sexual monomorphisms and dimorphisms do not reflect the phylogenetic relationships of the species concerned. The three species *D. simulans*, *D. mauritiana* and *D. sechellia* are closely related and members of the sister group of *D. melanogaster*. In *D. simulans* and *D. mauritiana*, both sexes have (*Z*)-7-tricosene (Cobb and Jallon, 1990), whereas *D. sechellia* is a sexual dimorphic species, males having (*Z*)-6-tricosene, a position isomer of (*Z*)-7-tricosene and females (*Z,Z*)-7,11-heptacosadiene (Cobb et al., 1989). Sex pheromones identified by using synthetic monoene or dienes are (*Z*)-11-pentacosene (C_{25}) of *D. virilis* (Oguma et al., 1992; Doi et al., 1996), (*Z,Z*)-5,27-tritriacontadien ($C_{33:2}$) of *D. pallidosa* (Nemoto et al., 1994) and (*Z,Z*)-5,25-hentriacontadien ($C_{31:2}$) of *D. ananassae* (Doi et al., 1997). Cuticular hydrocarbons of *Drosophila* were reviewed by Ferveur (2005).

Males' cuticular hydrocarbons are species specific and sexual dimorphisms are found in some species. Therefore, cuticular hydrocarbons of males can act as a sex pheromone. In *D. melanogaster*, (*Z*)-7-tricosene, a major component of cuticular hydrocarbons of males, affects mating of females (Grillet et al., 2006). Mating was enhanced by larger amounts of (*Z*)-7-tricosene from males with a GS10164 insertion driven by GAL4 compared with reduced (*Z*)-7-tricosene from $desat1^{1573-1}$ mutant males. Females whose antennae were surgically removed showed reduced mating, suggesting that females perceive male sex pheromones via antennae.

7. Mounting position and copulation

Spieth (1952) named two mounting positions, forward and rearward, and unnamed for a few exceptional species. The forward position is a type observed in *D. melanogaster*. A male places his forelegs at the base of the wings of a female or grasps her

thorax with his forlegs, grasps her abdomen with his middle legs, and lays his hindlegs on the substrate or grasps her abdomen. This mounting position seems to be primitive. The other type, the rearward position, is thought to be derived and seen in most species of the subgenus *Drosophila* and some species of the subgenus *Sophophora*. A male grasps a female's abdomen or wing tips with his forlegs and grasps her abdomen with his middle and/or hindlegs. Although there have been many reports describing the courtship behaviour, the mounting position is poorly described and clear descriptions were not found in many cases.

Positions of mounting and copulation are symmetric, that is, the axis of both sexes coincides. However, in *D. melanogaster*, the removal of a single sensillum of male genitalia affects the mating position and results in a contralateral asymmetry in the male's mating posture (Acebes et al., 2003). Although this sensillum affects the symmetry of the mounting position, the male seems to mount in the forward position, judging from the photograph in Figure 2 in Acebes et al. (2003).

Copulation or intromission is achieved before or after a male mounts a female. In the description of Spieth (1952), all species belonging to the subgenus *Drosophila* are classified as intromission after mounting, or no description. Most species of subgenus *Sophophora* are in the intromission before mounting or at the same time. Exceptional species in *Sophophora* are those belonging to the *montium* subgroup, *D. auraria*, *D. rufa* and *D. montium*, and *D. ananassae* of the *ananassae* subgroup; they are the species of the intromission after mounting. Copulation after mounting was observed in other species (*D. serrata*, *D. birchii*, *D. lini*, *D. ohnishii* and *D. ogumai*) of the *montium* subgroup (Hoikkala and Crossley, 2000; Wen et al., 2011).

Spieth (1952) suggested that there is a relationship between the male's grasping of the female and the presence of spines that species of the *immigrans* group have on the inside of the fore femur. Similar spines on the fore femur are also found in the genus *Zaprionus*. In the courtship behaviour of *D. silvestris*, a Hawaiian *Drosophila*, Carson (2002) argued for the importance of tactile stimulus from the male. The male of *D. silvestris* actively drums the tibial bristles (cilia) on the dorsal surface of the female's abdomen. Males of *D. mojavensis* drum continuously upon the underside of the female's abdomen using their fore tarsi (Krebs and Bean, 1991), suggesting the importance of tactile stimuli as well as courtship songs in this species.

8. Conclusion

The female does not play a passive role in courtship as was pointed out in the first report of courtship behaviour in *Drosophila* (Sturtevant, 1915). Since the male behaviour is a response to female sex pheromones, a male may be evaluated by a female in his ability of wing vibrations and following (Ferveur and Cobb, 2010). Before mounting, a female moves slowly as if the courting male can easily approach her. In response to courtship songs, a female spreads both her wings when she accepts the courting male in some species (Ikeda et al., 1981; Oguma et al., 1996; Vuoristo et al., 1996). In contrast, sometimes the female moves rapidly and escapes from the male. The female often kicks the male and/or produces rejecting sounds when he attempts to copulate. Females of the *virilis* group produce a song when accepting a courting male. In species in which the males hold territories, females visit a territory or a lek to choose their partner. Decision making based on a male's visual signal is also shown in *D. suzukii* females. In species in which the males produce sounds during courtship, songs seem to be a critical signal affecting the female's decision making. The females do not learn how to choose a partner; decision making to choose partners and its species specificity are genetically determined (Piñeiro et al., 1993; Isoherranen et al., 1999a; Doi et al., 2001).

The general structure and/or overall shape of courtship songs are similar within closely related species but the song parameters differ. For example, between *D. melanogaster* and *D. simulans*, some song parameters, such as interpulse interval, intrapulse frequency and sine song frequency, differ. In some cases, a gain or loss of song type is found. In the *melanogaster* subgroup, males emit a pulse song and a sine song, but *D. sechellia*, *D. yakuba* and *D. santomea* do not have a sine song. In the *virilis* group, male songs are polycyclic and females also sing. In contrast, a different but similar structure is found in distantly related species. The males of *D. lini* and related species in the *kikkawai* complex produce pulse and sine songs, although the overall shape of the courtship song of the *lini* clade is different from that

of *D. melanogaster*. In most cases, songs are species specific in terms of parameters (Table 1). In the case of closely related species that share similar values of sound parameters, they are allopatrically distributed (Tomaru et al., 1994a; Wen et al., 2011). Based on circumstantial evidence, courtship songs play an important role in sexual isolation between sympatric species and could be shaped by the process of reinforcement and/or reproductive character displacement.

Courtship songs are related not only to species recognition between species but also to sexual selection within species. Female choice based on the male's signal is observed, but it is not easy to figure out the evolutionary cause of female choice. Cost of mating is reported in *Drosophila* (e.g., Ueyama and Fuyama, 2003); however, it is poorly known how the females evaluate the quality of the courting males based on the male's signal (Chapman, 2006). Experiments were carried out to compare mating frequencies between intact males and males whose wings were partly removed in *D. melanogaster* (Robertson, 1982), *D. littoralis*, *D. montana* and *D. ezoana* (Hoikkala and Aspi, 1993). Females preferred intact or long-winged males better. Examination with wild caught females also showed preference to males that produced short pulses with high frequency songs (Aspi and Hoikkala, 1995). Variations are observed in male song parameters and in mate preference of females within species (Ritchie et al., 2005). Playback tests using artificially synthesised songs revealed that *D. montana* females preferred songs with a short pulse length and high pulse frequency (Ritchie et al., 1998). Furthermore, their preferences were not absolute but rather relative (Hoikkala and Aspi, 1993). Females choose males with high frequency songs (Hoikkala and Suvanto, 1999) and egg-to-adult survival rates positively correlate with the pulse frequency of the father's song in *D. montana* (Hoikkala et al., 1998); these results indicate that females obtain an indirect benefit by choosing males based on songs. Song divergence is not correlated with genetic divergence in the *D.* (*S.*) *willistoni* species complex, suggesting rapid evolution of song parameters (Gleason and Ritchie, 1998). Sexual selection seems to contribute, at least partly, to the evolution of species specific courtship songs.

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An evolutionary view on courtship behavior of *Drosophila*: From a comparative approach

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Courtship behavior is highly divergent in Diptera, especially in *Drosophila*. The typical courtship of *Drosophila melanogaster* is an elaborate repertoire including elements of orienting, tapping, scissoring, circling, wing-vibrating, licking and attempting to copulate, in which males and females send and receive visual, chemical, tactile and acoustic signals from each other. The divergence of courtship behavior reflects the phylogeny to some extent. Loss or gain of certain elements, which cause qualitative differences in courtship behavior among species, are very common in *Drosophila*. On the other hand, rapid quantitative changes in sexual signals tend to occur between sympatric, closely related species but not between allopatric, close relatives. In this review, we assemble available information on courtship behavior, compare its behavioral elements across 135 species of 30 species groups from three genera of the Drosophilidae, excepting the Hawaiian picture-wing *Drosophila* with a bizarre courtship pattern, and discuss functions of each element in relation to its loss or gain and possible evolutionary mechanisms underlying the sexual signal divergence.

1. Introduction

Mating success mainly relies on Specific Mate Recognition System (SMRS). Every sexually reproducing species possesses its own distinct SMRS that controls the exchange of sensory information sent and received by both sexual partners during interplay (Paterson, 1985). *Drosophila* courtship is an elaborate ritual which involves specific types of activity (certain elements), such as orienting, tapping, scissoring (scissor-like opening and closing of both wings without courtship song), circling, wing vibrating (one wing or both wings rapidly up and down with production of courtship song), licking and attempting to copulate, from both sexes. Visual, chemical and acoustic signals derived from these certain elements make up SMRS and the divergence of each element is the important factor contributing to premating isolation and speciation.

Courtship behavior is one of characters which have derived from the ancestor and then diverged to be adapted to environmental changes or evolved through sexual selection and speciation. Mating in flight is the ancestral Dipteran behavioral pattern and is common in most primitive families such as mosquitoes, of which males have the auditory plumose antenna sensitive to the flight tone of the female and the enlarged specialized eye to capture the female (Downes, 1969). However, such behavior is rare in the derived Acalypteratae with the dichoptic eyes (McAlpine and Munroe, 1968). In the Drosophilidae, mating behavior is highly interactive between males and females especially before mounting. The drosophilid matings typically occur on some types of food substrates, associated with more or less species-specific courtships or epigamic displays making use of posture, movements or display of patterns before attempting to mount and copulate (Spieth, 1952). The great variety of environmental and food conditions has mainly determined the way in which flies can meet and mate, and explains the diversity of mating behaviors (Wicker-Thomas, 2007). Such a drosophilid mating system appears to possess adaptive advantages: males could ascertain many females with small expenditure of time and energy; unreceptive females could repel males during feeding; receptive females could excise sexual selection on several

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males. The diurnal feeding shortly on a small discrete food mass is the key factor responsible for the selective pressure on the evolution of this courtship pattern (Spieth, 1974).

Certain elements of mating behavior are widespread across various species, but it is also obvious that qualitative as well as quantitative interspecific differences exist. A few, if any, quantitative differences are observable between closely related species, while more distantly related species invariably display qualitative differences. In *Drosophila* courtship, the male is primarily responsible for sexual isolation and the female for sexual selection (Spieth, 1952, 1974; Brown, 1965). Closely related species or races derived from allopatric localities are often less sexually isolated than those derived from sympatric sites, the former displaying an identical courtship pattern but the latter distinct ones (Ehrman, 1965; Spieth, 1978). This suggests the reinforcement of divergence in courtship signals between sympatric species/races (Chang and Miller, 1978; Wen et al., 2011). To understand the evolutionary mechanism of courtship behavior, i.e., how and why the relevant characters have reached their present forms, one must combine comparative ethological works with studies on the function, origin and genetics of behavior (Hinde and Tinbergen, 1958; Brown, 1965). It is important to examine roles of courtship in mate selection and reproductive isolation. The initial observations of *Drosophila* mating behavior were made by geneticists interested in evolutionary problems (Ewing, 1983). Before Spieth's (1952) pioneer work on courtship behavior of 101 *Drosophila* species belonging to 21 species groups, mating behavior of *D. melanogaster*, *D. pseudoobscura*, *D. persimilis*, *D. affinis*, *D. algonquin*, and some species of the *D. willistoni* species group and the *D. virilis* species group had already been documented by various investigators (Sturtevant, 1915; Spieth, 1947, 1948; Miller, 1950; Spieth, 1951). Hitherto, courtship behaviors of more than 135 species belonging to 30 species groups and some Hawaiian picture-wing *Drosophila* species have been observed by many investigators. Thus accumulated information makes comparison of courtship behaviors possible across the largest genus *Drosophila* in the Drosophilidae. Tomaru and Yamada (2011) thoroughly reviewed courtship behaviors of *Drosophila*, mainly focusing on their courtship songs of acoustic stimuli. In this review, therefore, we provide information on other behavioral elements, especially those related to visual and chemical stimuli, from a viewpoint of how certain elements of courtship have diverged along the evolutionary process. Since in *Drosophila* courtship males are more active and play vital roles for successful mating, we concentrate mainly on male courtship. The elements present or absent in each species are summarized in Table 1 from selected references, excepting some others having failed to observe some behaviors in some species by careless observation or using different strains (Ewing, 1983). We exclude Hawaiian picture-wing *Drosophila* species from the comparison in this review, because an aberrant type of courtship behavior and advertising behavior in a small territory or lek was displayed by these endemic species (Spieth, 1974).

2. Behavioral elements and their functions in *Drosophila* courtship

Apart from endemic Hawaiian picture-wing *Drosophila* species which mate on their leks or territories, most *Drosophila* species display courtship during feeding on a food mass. *Drosophila* courtship is very diverse among species, and requires visual, acoustic, and chemical cues (Wicker-Thomas, 2007). Each species possesses a species-specific repertoire of signals associated with unique movements. Homologous signals are quite different between distinct species, but similar, with some quantitative differences, between closely related species.

Drosophila male courtship is commonly composed of the elements of orientation, tapping, wing flicking, wing waving, wing semaphoring, wing scissoring, wing vibration, leg vibration, leg rubbing, circling, licking, mounting and countersignaling (Spieth, 1974). Each element has its function for leading to successful copulation (Spieth, 1952, 1974; Ewing, 1983). Males and females send and/or receive several stimuli, such as visual, chemical, tactile or acoustic ones, from each other during courtship. Males' dancing by flicking, waving, scissoring or rowing of one or two wings and circling is a visual language to females; chemical signals and tactile stimuli are transmitted between males and females by males' tapping and licking on females; acoustic signals, probably visual signals as well, are produced by vibrating one or two wings. In many species, the role of visual signals is less species-

specific than the roles of chemical or acoustic signals (Markow and O'Grady, 2005).

2.1 Courtship elements related to visual stimuli

Visual stimuli are one of the major factors in the initiation of courtship (Spieth, 1952). At the beginning of courtship, a male orients a moving female and then taps her. Few *Drosophila* females are so receptive that they will accept a male as soon as he taps (Manning, 1960). After tapping, the male performs some wing display behaviors or circling to let the female slow down. Wing displays include wing waving, flicking, scissoring, rowing, or vibrating which probably produces both visual and acoustic signals. Thus, such visual signals are sent from males to females and should be received by females under light.

2.1.1 Light dependency

Light is required for *Drosophila* to receive visual stimuli from environmental factors or individuals of conspecific or alien species. In *Drosophila*, some species fail to mate or reduce mating success without light, while some others are able to mate in the darkness; i.e., the former is dependent but the latter independent on light for mating. It has been well documented that the extent of light-dependency for mating varies among *Drosophila* species.

According to the classification by Grossfield (1971), light-independent species (8 spp.) are less than light-dependent ones (12 spp. being somewhat light-dependent and 22 spp. completely dependent). For the light-independent species, such as *D. melanogaster*, *D. busckii*, *D. ananassae*, *D. montium*, *D. pseudoobscura*, *D. affinis*, *D. funebris*, *D. virilis*, *D. hydei*, *D. replete* and *D. immigrans*, visual stimuli by wing display or circling might be dispensable but courtship might more rely on chemical stimuli by tapping/licking and/or acoustic stimuli by wing vibration (Table 1). For some completely light-dependent species, such as *D. subobscura*, *D. munda*, *D. palustris*, *D. phalerata* and *D. guttifera*, which produce no acoustic stimuli, courtship might mainly rely on visual stimuli by wing display and/or circling and subsequently following elements (Table 1). Other more or less light-dependent species use both visual and chemical/acoustic stimuli for courtship.

2.1.1.1 *D. subobscura*

Drosophila subobscura is one of species which completely depend on light for courtship (Grossfield, 1971). While a male and a female are moving face to face, the male gradually opens his wings, and spreads them when he stops to move. This "wing-dance" is the only effective courtship element in this species. Brown (1965) conducted two experiments to test the importance of visual stimuli of this "wing-dance" for mating success. The first was to remove the male wings completely or the distal half of them. The results indicated that the number of successful mating pairs was significantly reduced in both wing removal treatments, which made females walk away from the wing-removed males. The second was to show a black spherical "Plasticine" model moving side by side or an etherized female to a virgin male. In response to the moving experimental object, some virgin males tapped, orientated, wing-danced and even tried to copulate with the moving model. Some virgin males ignored the etherized female until she began to move. Thus, the first experiment revealed that the display of wing opening and spreading was important for the female to keep staying with the male. The second experiment suggested that a side-by-side moving article was the cue for male to pay attention to court. Interestingly, the wing vibration is very rare in this species, suggesting that any acoustic signal is not used as a cue to stimulate its partner. *Drosophila subobscura* is the only species that does not sing any courtship songs but performs the unique wing-dancing in the *D. obscura* species group (Table 1). *Drosophila subobscura* occurs in a variety of habitats from woodlands, pastures, even to moorlands. It has a good deal of genetic heterogeneity, and this might serve to adapt it to a wide variety of habitats (Brown, 1965). There is a light-independent strain selected from a geographic population. This strain shows the totally modified courtship lacking the wing-dance; the males rape the females without preceding courtship. The females seem to be rather passive and do not resist atypical copulation attempts by the males. The behavioral differences between the light-dependent and -independent strains are genetically determined by a polygenic system involving all four autosomes but not the X-chromosome (Pinsker and Doschek, 1980). It seems likely that this genetically determined variation of courtship seen in wild populations is the consequence of natural selection under different environments.

2.1.1.2 The *D. melanogaster* species complex

Drosophila melanogaster is the only light-

Table 1: Elements of male courtship behavior in the Drosophilidae

Genus	Subgenus	Species-group	Species-subgroup	Species	Before mounting							Being or after mounting	(A±B)** signal divergence between closely related species	References***
					Chemical stimuli		Visual stimuli			Acoustic stimuli	Male-male courtship	Acoustic stimuli		
					tapping	licking	circling	scissoring/flicking/waving/rowing	light dependency	wing vibration*		wing vibration		
Scaptodrosophila		*victoria*		*S. victoria*	+	−	−	−	N/A	V3-5	+	−	N/A	1
		rufifrons		*S. lebanonensis*	+	−	−	−	N/A	V3-5	+	−	N/A	1
		latifasciaeformis		*S. latifasciaeformis*	+	+	+	−	N/A	V90	−	−	N/A	1
Hirtodrosophila				*H. duncani*	+	−	−	−	N/A	−	−	−	N/A	1
Drosophila	*Sophophora*	*saltans*	*saltans*	*D. prosaltans*	+	+	+	−	N/A	V20-40, V90	−	−	N/A	1
				D. saltans	+	+	+	−	N/A	V90	−	−	N/A	1
			sturtevanti	*D. rectangularis*	+	+	+	−	N/A	V20-40	−	−	N/A	1
			elliptica	*D. emarginata*	+	+	+	F	N/A	−	−	−	N/A	1
		willistoni	*willistoni*	*D. willistoni*	+	+	−C	F, S	N/A	V70-90	−	−	pulse song	4; 1; 5
				D. equinoxialis	+	+	−C	F	N/A	V70-90	−	−		4; 1; 5
				D. paulistorum	+	+	−C	F	N/A	V70-90	−	−		1; 5
				D. tropicalis	+	+	−C	F	N/A	V70-90	−	−		1; 5
			bocainensis	*D. sucinea*	+	+	−C	F	N/A	V70-90	−	−	pulse song	4; 1
				D. capricorni	+	+	−C	F	N/A	V70-90	−	−	N/A	4; 1
				D. fumipennis	+	+	−C	W, S	N/A	−	−	−	N/A	4; 1
				D. nebulosa	+	+	−C	W, S	N/A	−	−	−	N/A	4; 1
		melanogaster	*melanogaster*	*D. melanogaster*	+	+	+	S (rare)	I	V90	+	−	(mel+sim) locomotory activity/wing display/pulse song/hydrocarbon	1; 6; 7; 8; 3
				D. simulans	+	+	+	S (many), R1/2	II	V90 (rare)	+	−		1; 6; 7; 8; 3
				D. sechellia	+	+	+	S, R1	II	V	N/A	−		8;
				D. mauritiana	+	+	+	S, R2 (many)	II	V90 (rare)	+	−		9; 8
				D. yakuba	+	+	+	S, R2	II	V	N/A	−	(yak+san) pulse song	8; 10
				D. santomea	+	+	+	S, R2	II	V	N/A	−		11; 10
				D. teissieri	+	+	+	S, R2	II	V	N/A	−	N/A	8
				D. erecta	+	+	+	S	II	V	N/A	−	N/A	8
				D. orena	+	+	+	S	II	V	N/A	−	N/A	8
			suzukii	*D. biarmipes*	+	+	−	S	N/A	V	N/A	−	N/A	12
			elegans	*D. elegans*	+	−	+	W180	N/A	−	+	−	(ele+gun) circling/wing display	13
				D. gunungcola	+	−	−	W30	N/A	−	+	−		13
			takahashii	*D. takahashii*	+	−	+	W	II	V90	+	−	N/A	14; 1; 3
			ananassae	*D. ananassae*	+	−	+	S	I	V5-15	+	−	(ana+pal) pulse song/hydrocarbon	14; 1; 3; 15; 16
				D. pallidosa	+	−	+	S	N/A	V50-90	+	−		17; 15; 16; 18
				D. malerkotliana	+	+	+	S	N/A	V	N/A	−	N/A	19
				D. bipectinata	+	−	N/A	N/A	N/A	V5-15	N/A	−	N/A	20
			montium	*D. auraria*	+	−	−	−	II	rare	+	+	(aur+tri+bia+sub) (qua-tri) pulse song	14; 1; 21; 22
				D. triauraria	+	−	−	−	II	rare	+	+		21; 22
				D. biauraria	+	−	−	−	III	rare	+	+		21; 22
				D. subauraria	+	−	−	−	N/A	rare	+	+		23
				D. quadraria	+	−	−	−	N/A	rare	+	+		23
				D. rufa	+	−	−	−	II	−	+	+	N/A	14; 1; 3
				D. montium	+	−	−C	−	I	−	+	+	N/A	14; 1; 3
				D. serrata	−	−	rare	−	N/A	rare	+	+	(ser+bir) pulse song	24; 25
				D. birchii	−	−	rare	−	N/A	rare	+	+		24; 25
				D. lini	−	−	−	−	N/A	−	+	+	(lin-ogu) sing song frequency between ohn and lin/ogu	26; 27
				D. ogumai	−	−	−	−	N/A	−	+	+		26; 27
				D. ohnishii	−	−	−	−	N/A	−	+	+		26; 27
				D. kikkawai	−	−	−	−	N/A	−	+	+	(kik+leo)/(leo+boc)	28; 42
				D. leontia	−	−	−	−	N/A	−	+	+		28; 42
				D. bocki	−	−	−	−	N/A	−	+	+		28; 42

Drosophila	*Sophophora*	*obscura*	*pseudoobscura*	*D. pseudoobscura*	+	−	−	F	I	V90	+	−	(pseu+per) pulse song	29; 3; 30
				D. persimilis	+	−	−	F	II	V90	+	−		1; 29; 3; 30
				D. miranda	+	−	−	F	N/A	V90	+	−	N/A	1; 29
			obscura	*D. obscura*	−	−	−	F	N/A	V135	N/A	−	N/A	29
				D. tristis	−	−	−	F	N/A	V90	N/A	−	N/A	29
				D. bifasciata	+	−	+	F	N/A	V90-100	N/A	−	N/A	29
				D. ambigua	−	−	−	F	N/A	V90	−	−	N/A	29
			subobscura	*D. subobscura*	+	−	+	W (many)	III	−	+	−	N/A	1; 29; 3
			affinis	*D. affinis*	+	−	−C	F	I	V90	+	−	timing of singing/song type	31; 1; 32; 33
				D. algonquin	+	−	+	F	III	V90	+	+		31; 1; 32
				D. asteca	+	−	+	F	N/A	V90	+	−	N/A	1; 32
				D. athabasca	+	−	+	F, W	N/A	V40-90	+	−	N/A	1; 32
	Dorsilopha			*D. busckii*	+	+	−C	F	I	V90	+	−	N/A	1; 2; 3
	Phloridosa			*D. lutzii*	−	−	−	−	N/A	−	N/A	−	N/A	1
	Drosophila	*polychaeta*		*D. polychaeta*	+	+	−	−	N/A	−	+	−	N/A	1
		virilis	*virilis* phylad	*D. virilis*	+	+	−C	F	I	V10-15	+	−	pulse song	35; 1; 3; 36
				D. novamexicana	+	+	−C	−	N/A	V10-15	+	−		1; 36
				D. a. americana	+	+	−C	F	N/A	V10-15	+	−		35; 1; 36
				D. a. texana	+	+	−C	−	N/A	V10-15	+	−		1; 36
			montana phylad	*D. lacicola*	+	+	−C	−	N/A	V10-15	+	−	pulse song	1; 36
				D. montana	+	+	−C	−	N/A	V10-15	+	−		1; 36
		melanica		*D. melanica*	+	+	−C	−	III	V5-10	+	−	N/A	1; 3
				D. paramelanica	+	+	−C	−	N/A	V5-10	+	−	N/A	1
				D. melanura	+	+	−C	−	N/A	V5-10	+	−	N/A	1
				D. micromelanica	+	+	−C	−	N/A	V5-10	+	−	N/A	1
				D. nigromelanica	+	+	−C	−	N/A	V5-10	+	−	N/A	1
		robusta		*D. robusta*	+	+	−	−	II	V10-15	+	−	N/A	1; 3
		repleta	*hydei*	*D. hydei*	+	+	−	−	I	V15	+	−	N/A	1; 3
				D. nigrohydei	+	+	−	−	N/A	V15	+	−	N/A	1
			repleta	*D. repleta*	+	+	−	−	I	V15	+	−	N/A	1; 3
				D. neorepleta	+	+	−	−	N/A	V15	+	−	N/A	1
				D. fulvimacula	+	+	−	−	N/A	V15	+	−	N/A	1
				D. limensis	+	+	−	−	N/A	V15	+	−	N/A	1
			mulleri	*D. pegasa*	−	−	−	−	N/A	−	+	−	N/A	37; 38
				D. aldrichi	+	+	−	−	N/A	V15	−	−	(ald+mul) N/A	1
				D. mulleri	+	+	−	−	II	V15	−	−		1; 3
				D. buzzatii	+	+	−	−	N/A	V15	−	−	N/A	1
				D. hamatofila	+	+	−	−	N/A	V15	−	−	N/A	1
				D. longicornis	+	+	−	−	N/A	V30-40	−	−	N/A	1
				D. meridiana rioensis	+	+	−	−	N/A	−	−	−	N/A	1
				D. mojavensis	+	+	−	−	N/A	V15	−	−	(moj+ari) female	1; 39; 40
				D. arizonae	+	+	−	−	N/A	V15	−	−	acceptability	39
				D. ritae	+	+	−	−	N/A	V15	−	−	N/A	1
				D. anceps	+	+	+	F	N/A	V15	−	−	(anc+leo) elements lose/gain, pulse song	1; 41
				D. leonis	−	+	+	rare	N/A	V15	−	−		41
				D. nigrospiracula	−	+	−	F	N/A	V15-20	−	−		41
				D. eremophila	+	+	−	−	N/A	V70-80	−	−		41
				D. mettleri	+	+	−	−	N/A	V70-80	−	−	allopatric	41
				D. micromettleri	+	+	−	−	N/A	V70-80	−	−		41
				D. nigricruria	+	+	−	−	N/A	V15	+	−	N/A	1
			mercatorum	*D. m. mercatorum*	+	+	−	−	N/A	V15	−	−	N/A	1
				D. peninsularis	+	+	−	−	N/A	V15	−	−	N/A	1
		annulimana		*D. gibberosa*	+	+	−	−	III	V5	+	−	N/A	1; 3
		carbonaria		*D. carbonaria*	+	+	−C	F	N/A	−	+	−	N/A	1
		nannoptera		*D. nannoptera*	+	+	−	−	N/A	V20-30	+	−	N/A	1
				D. acanthoptera	+	+	+	−	N/A	V75	+	−	N/A	1

Genus	Subgenus	Species-group	Species-subgroup	Species	Before mounting							Being or after mounting	(A±B)** signal divergence between closely related species	References***
					Chemical stimuli		Visual stimuli			Acoustic stimuli	Male-male courtship	Acoustic stimuli		
					tapping	licking	circling	scissoring/flicking/waving/rowing	light dependency	wing vibration*		wing vibration		
Drosophila	*Drosophila*	*tumiditarsus*		*D. repletoides*	+	−	−	−	N/A	−	−	−	N/A	1
		immigrans	*immigrans*	*D. immigrans*	+	−	+	F, S	I	−	−	−	N/A	1
			nasuta	*D. sulfurigaster*	+	−	+	W	N/A	−	+	−	N/A	1
		funebris	*funebris*	*D. funebris*	+	+	−C	F	I	V	+	−	N/A	1; 3; 43
			macrospina	*D. subfunebris*	+	+	−C	−	N/A	V	+	−	N/A	1; 43
				D. m. macrospina	+	+	−C	−	N/A	V	+	−	N/A	1; 43
				D. m. limpiensis	+	+	−C	−	N/A	V	+	−	N/A	1; 43
				D. m. ohioensis	+	+	−C	−	N/A	−	+	−	N/A	1
				D. trispina	+	−	−C	F	N/A	−	+	−	N/A	1
		cardini	*cardini*	*D. cardini*	+	+	+	−	N/A	−	−	−	N/A	1
				D. cardinoides	+	+	−	F	N/A	−	−	−	N/A	1
				D. neocardini	+	+	−C	W, F	N/A	−	+	−	N/A	1
				D. polymorpha	+	+	−	−	N/A	V	N/A	−	N/A	1
		quinaria		*D. innubila*	+	−	−	F	N/A	−	+	−	N/A	1
				D. munda	+	+	−	−	III	−	+	−	N/A	1; 3
				D. occidentalis	+	−	−	F	II	−	+	−	N/A	1; 3
				D. suboccidentalis	+	−	−	F	N/A	−	+	−	N/A	1
				D. palustris	+	+	−	F	III	−	+	−	N/A	1; 3
				D. subpalustris	+	−	−C	−	II	−	+	−	N/A	1; 3
				D. quinaria	+	−	−	−	N/A	−	+	−	N/A	1
				D. tenebrosa	+	+	−	F	N/A	−	few	−	N/A	1
				D. phalerata	+	−	−C	F	III	−	+	+	(pha+tra) timing of singing/	1; 3; 34
				D. t. transversa	+	−	−C	−	N/A	V20-30	+	−	pulse song	1; 34
				D. t. subquinaria	+	+	−C	−	N/A	−	+	−	N/A	1
		guttifera		*D. guttifera*	+	+	−	−	III	−	+	−	N/A	1; 3
		testacea		*D. putrida*	+	+	−C	S	N/A	−	+	−	N/A	1
		tripunctata		*D. tripunctata*	+	−	−C	F	III	V75-90	+	−	N/A	1; 3
				D. crocina	+	+	−C	−	N/A	V15-20	+	−	N/A	1
		guarani	*guarani*	*D. subbadia*	+	+	−C	−	III	V5	−	−	N/A	1; 3
		rubrifrons	*uninubes*	*D. parachrogaster*	+	+	−C	−	N/A	V90	+	−	N/A	1
		castanea		*D. castanea*	+	+	−C	−	N/A	V5	−	−	N/A	1

+: present; −: absent; N/A: no information available; −C: males circle non-receptive females; rare: males rarely display the behavior; many: male's behavior is abounding; W: wing waving, F: wing flicking, S: wing scissoring, R1: one wing rowing, R2: two wings rowing; I: light-independent, II: somewhat light-dependent, III: complefely light-dependent.

* The number indicates the degree of wing extension from the body axis.

** (A+B) indicates sympatric species A and B; (A-B) indicates allopatric species A and B.

*** 1. Spieth (1952); 2. Bixler et al. (1992); 3. Grossfield (1971); 4. Spieth (1947); 5. Ritchie and Gleason (1995); 6. Manning (1960); 7. Ewing et al. (1968); 8. Cobb et al. (1989); 9. Robertson (1983); 10. Blyth et al. (2008); 11. Llopart et al. (2002): 12. Hegde et al. (2005); 13. Yeh et al. (2006); 14. Spieth and Hsu (1950); 15. Yamada et al. (2002a); 16. Yamada et al. (2002b); 17. Futch (1973); 18. Yamada et al. (2008); 19. Hegde and Krishna (1997); 20. Ewing and Bennet-Clark (1968); 21. Oguma et al. (1984); 22. Oguma et al. (1996); 23. Tomaru and Oguma (1994); 24. Hoikkala et al. (2000); 25. Hoikkala and Crossley (2000); 26. Oguma et al. (1995); 27. Wen et al. (2011); 28. Baimai et al. (1980); 29. Brown (1965); 30. Noor and Aquadro (1998); 31. Miller (1950); 32. Chang and Miller (1978); 33. McRobert and Tompkins (1987); 34. Neems et al. (1997); 35. Stalker (1942); 36. Hoikkala and Lumme (1984); 37. Wasserman et al. (1971); 38. Wasserman and Zweig (1991); 39. Markow (1981a); 40. Krebs and Bean (1991); 41. Alonso-Pimentel et al. (1995); 42. Li and Wen (unpublished data); 43. Ewing (1979).

independent species in this species complex. *Drosophila simulans*, *D. mauritiana* and *D. sechellia* depend on light to more or less extent. The decrease of mating frequency in darkness is the least in *D. simulans*, the most in *D. sechellia*, and intermediate in *D. mauritiana* (Spieth and Hsu, 1950; Grossfield, 1972; Robertson, 1983; Sakai et al., 1997). The normal courtship of *D. melanogaster* involves shortly orient-front behavior but abounding wing vibration (Manning, 1960; Markow and Hanson, 1981). Markow and Hanson (1981) dissected the courtship of *D. melanogaster* by multivariate analyses and found out that the orient-front behavior during the circling frequently happened in unsuccessful or long-bout courtships to give more visual signals for exciting the unreceptive female. In contrast, the visual information, such as scissoring, is dominant in its sympatric species, *D. simulans*, which displays more scissoring and spends less time on wing vibration. *Drosophila mauritiana*, most closely related to *D. simulans*, is light-dependent as well, spending less time for wing vibration but longer time for sending visual stimuli (Cobb et al., 1989), although it uses wing rowing rather than scissoring as a display.

The light-dependency varies also between strains of *D. simulans*. Sakai et al. (1997) found that one strain from Ogasawara, Japan was light-independent like as *D. melanogaster*. The intraspecific difference of mating frequency in darkness depends on genetic differences in the male-specific sexual behavior rather than in the locomotor or mating activity (Sakai et al., 1997).

2.1.1.3 The *D. auraria* species complex

Mating behavior of three species, *D. auraria*, *D. triauraria* and *D. biauraria*, in the *D. auraria* species complex is very similar, lacking wing-display and front-circling: a male approaches and taps a female, and then circles to her rear, and makes a running lunge when her position is appropriate to mount; having mounted and grasped the female, the male extends one wing to 90° and vibrates it, and at the same time attempts to copulate. The light-dependency varies to some extent between the species: *D. biauraria* is completely light-dependent, while *D. auraria* and *D. triauraria* are somewhat light-dependent (Spieth and Hsu, 1950; Oguma et al., 1984; Oguma et al., 1996).

Oguma et al. (1996) used an infrared camera to record courtship behavior of the *auraria* complex under darkness, and found that tapping and subsequent elements triggered by visual signals were rarely observed under darkness. However, when flies were in close contact, males mounted females and attempted to copulate. A picture in the paper showed that a male of *D. auraria* copulating with a female vibrated his wing in darkness, and that three females near the copulating couple spread their wings in response to the wing vibration of the male. Obviously, acoustic signals should have induced female's receptive response under darkness in this species. This finding indicates that *D. auraria* can mate in the dark if partners are in close contact, although Grossfield (1971) classified this species as a completely light-dependent one.

2.1.2 Evolution of elements related to visual stimuli

Grossfield (1971) divided *Drosophila* into three classes by investigating insemination rate under light and darkness: Class I is light-independent, with species distributed worldwide or over wide geographic regions; Class II and III are somewhat and completely light-dependent, respectively, with species distributed in limited regions, except for the cosmopolitan *D. simulans* (Grossfield, 1972). Two distantly related species, *D. simulans* and *D. subobscura*, have evolved the light-dependent feature independently (Spieth and Hsu, 1950). Males of either species do not use acoustic cue but mainly wing display during courtship. Males send visual signals to females by vigorous wing display: wing-dance in *D. subobscura* and scissoring in *D. simulans*. Females have to receive these messages under light. This feature may have evolved similarly, even though independently, probably through the loss of acoustic signals in males or degeneration of hearing sensory in females, and the enhancement of visual signals by wing-dance, scissoring and/or circling in males or the gain of susceptive visual sensory in females, in these two unrelated species.

Visual stimuli are less used in the *D. montium* species subgroup. Species in this subgroup display similar courtship behavior without wing display or circling before mounting, but use more acoustic signals by wing vibration after mounting. This is a very unique and typical courtship pattern in this subgroup. The light-dependency varies among species in this subgroup: *D. biauraria* is completely dependent, *D. auraria* and *D. triauraria* highly dependent, *D. rufa* less dependent, and *D. montium* independent (Gross-

field, 1972; Oguma et al., 1996). In this subgroup, the light-dependency might have evolved as the consequence of adaptations to environments or ecological habitats where they are living.

2.2 Elements related to chemical stimuli

Two elements, tapping and licking, are associated with chemical stimuli, although tactile stimuli might be involved as well. Courtship of many species in the *melanogaster* subgroup is initiated by male's tapping on female with his fore tarsi where chemoreceptors are located, and then other elements, such as following, circling, wing display and wing vibration, take place. After wing vibration, the male licks the female's genitalia with his proboscis and then attempts to copulate (Ewing and Bennet-Clark, 1968). Spieth (1952) amputated the male fore tarsi in four species of the *virilis* group and confirmed that stimuli were received by the male via his fore tarsi and that differences existed in this factor between strains of the same species. When males tap other males, some species, such as those of the *D. mulleri* species subgroup of the *D. repleta* species group and the *D. saltans* and *D. willistoni* species groups, stop courtship immediately (Table 1), suggesting that they discriminate between conspecific males and females based on chemical/tactile signals obtained by tapping. However, some other species continue male-male courtship even after tapping. In *D. melanogaster*, tapping with correct gustatory inputs is necessary for males to induce the species-specific hallmark of unilateral wing vibration, because the flies deprived of gustatory inputs become to perform bilateral wing extension during courtship (Koganezawa et al., 2010). Thus, the pheromone input by tapping from the foreleg of courting male shapes the courtship song pattern. The licking action after wing vibration of the male obviously stimulates the female. It is possible that chemical stimuli are involved in this action (Spieth, 1952). Tapping is, however, absent in many species of the *montium* and the *obscura* subgroups, and licking is totally omitted in all investigated species of these subgroups (Table 1). Commonly seen male-male courtships in the *montium* subgroup might be due to the lack of tapping and/or licking elements. Other elements, such as wing vibration after mounting, play a very important role in discriminating mates in this subgroup, for example in *D. lini*, *D. ogumai* and *D. ohnishii* (Wen et al., 2011).

The tapping and licking elements of courtship might have evolved along with female sex pheromones. The first *Drosophila* sex pheromone was identified from *D. melanogaster* (Antony and Jallon, 1982). Since then, a variety of cuticular hydrocarbon molecules have been identified from females and males in a number of *Drosophila* species (see Ferveur, 2005; for a review). Recently, Ferveur and Cobb (2010) have reviewed behavioral and evolutionary roles of cuticular hydrocarbons, and Wicker-Thomas and Chertemps (2010) have done the genetics and biosynthesis of them.

3. Timing of courtship: precopulatory courtship vs. copulatory courtship

The timing of male courtship should be an important factor to be considered for understanding the evolution of courtship behavior. Copulatory courtships, i.e., courtships occurring after intromission, are very common in insect (Eberhard, 1991). However, elaborated courtships in most *Drosophila* species are of typical precopulatory one. Only a few species of the *D. quinaria* species group and the *mulleri*, the *affinis* and the *montium* species subgroups have been reported to perform copulatory courtships with some variation (Miller, 1950; Spieth, 1952; Wasserman et al., 1971; Tomaru and Oguma, 1994; Neems et al., 1997; Wen et al., 2011).

3.1 *D. pegasa*

Drosophila pegasa is the first species which was reported to have no precopulatory courtship in *Drosophila*. When a male detects a female, he immediately climbs upon her abdomen and rides on her for a long period (Wasserman et al., 1971). The extended riding of male on female was called "grasping behavior" or "mate grasping" (Wasserman et al., 1971; Wasserman and Zweig, 1991; Gronlund et al., 2002). Females play only a passive role in mounting, but males can clearly distinguish mates, preferring females, upon initial grasping. Male-male grasping occurs when no female is present, but such a male usually releases his hold soon. It is still unknown what signals are used for males to differentiate between males and females, because the cuticular hydrocarbon composition is the same in both sexes (Gronlund et al., 2002). Males can distinguish even females reared on different types of food or from different localities, preferring females reared on the original host cactus media or from the

same local population, respectively (Wasserman and Zweig, 1991). In *D. pegasa*, this grasping behavior has evolved along with the complete loss of other elements that are commonly seen in the normal courtship of *Drosophila*.

3.2 *D. algonquin* and *D. phalerata*

Drosophila algonquin is a sibling species of *D. affinis* in the *affinis* subgroup of the *obscura* group. They are widely distributed in the United States east of the Rocky Mountains, with the overlapped range in the western Great Plains and in the northeastern States. Sex combs of *D. algonquin* are larger than those of *D. affinis* (Miller, 1950). In both species, courtship is usually very brief, being followed quickly by copulation. While males of *D. algonquin* sometimes continue to buzz during copulation, such a behavior is not found in *D. affinis* and other species of the *affinis* subgroup. Each burst of buzz was produced by vibration of both wings extended about 45° and lasted about a third of one second; 66 buzzes were counted in a copulation lasting for almost 5 min (Miller, 1950; Chang and Miller, 1978).

Drosophila phalerata feeds on fungi, usually together with *D. transversa*, *D. limbata* and *D. kuntzei* of the *quinaria* group in different combinations. All four species produced courtship songs by vibrating one or both wings in their courtships. Males of *D. phalerata* produce most of their songs during rather than prior to copulation, whereas males of the other three species remain silent during copulation. The song of *D. phalerata* consists of unstructured pulses with 260 Hz of carried frequency (Neems et al., 1997), being similar to the sine songs in the *D. lini* clade (Wen et al., 2011).

The copulatory courtship with wing vibration after mounting has independently evolved only once in *D. algonquin* and in *D. phalerata* among relatives of the *affinis* subgroup and of the *quinaria* group, respectively.

3.3 The *D. auraria* and the *D. serrata* species complexes

The *auraria* complex consists of four sympatric species, *D. auraira*, *D. biauraria*, *D. triauraria* and *D. subauraria*, and one allopatric species, *D. quadraria* (Kimura, 1987). The courtship behavior of five species is similar. When a male finds a female, he orients to her, then follows and taps her body. Wing vibration is observed during following in most cases. Males produce less courtship songs before copulation and at attempted copulation but more during copulation (Tomaru and Oguma, 1994). Thus, both precopulatory and copulatory courtships are performed, but with different intensities, by these species.

Drosophila birchii and *D. serrata* are sympatric sibling species which are endemic to Australia. Males of these two species court their females mainly during copulation, singing after mounting and during copulation. Females of both species discriminate against wing-removed conspecific males which do not sing (Hoikkala et al., 2000), strongly suggesting that their copulatory courtship song is an essential cue for mating success.

3.4 The *D. kikkawai* species complex

Three sibling species, *D. lini*, *D. ohnishii* and *D. ogumai*, form a clade in the *D. kikkawai* species complex of the *montium* subgroup (Wen, 2004). *Drosophila lini* and *D. ogumai* are allopatric, and *D. ohnishii* is distributed in the intermediate region, parapatrically with each of the other two species (Oguma et al., 1995; Zannat and Toda, 2002; Wen et al., 2011). Males follow moving flies and attempt to mount without tapping, circling or wing display before mounting. Immediately after mounting a female, the male extends either of his wings to 45–90° from the body axis, vibrates it, and attempts to copulate. Males produce sing songs with different frequencies, high (about 250 Hz) in the two allopatric species, *D. lini* and *D. ogumai*, but low (about 190 Hz) in *D. ohnishii* (Wen et al., 2011). Very interestingly, this difference in the sine song frequency exactly corresponds to the presence or absence of sexual isolation between the species; i.e., there is strong sexual isolation between the parapatric pairs of species, *ohnishii*/*lini* and *ohnishii*/*ogumai*, but no isolation between the allopatric pair, *lini*/*ogumai*. Playing back low frequency sine songs to *D. lini* and *D. ogumai* or high frequency songs to *D. ohnishii* has induced female repelling behavior (Li et al., in preparation), strongly suggesting that the frequency of sine song produced in copulatory courtship is an important sexual signal for mate recognition in these sibling species.

Three other sibling species, *D. kikkawai*, *D. leontia* and *D. bocki*, form another clade in the *kikkawai* complex (Wen, 2004). The distribution range of *D. leontia* overlaps with those of the other two species (Baimai et al., 1980). We observed their courtship behavior and found that they also displayed copulatory courtships like as the *lini* clade. Mounting of an

alien species male also induced female repelling behavior (Li et al., unpublished data).

4. Sexual signal divergence in sympatric closely related species

Natural selection strengthens sexual isolation in response to maladaptive hybridization following secondary contact of two species/subspecies/populations through the process known as reinforcement (Noor, 1999). Sexual isolation may result from divergence in sexual signals and receiver preferences between closely related species, so that speciation will be influenced by changes in signals and preferences (Butlin and Ritchie, 1994). Sexual signal divergence is sometimes seen in some courtship elements between sympatric closely related species: for example, in locomotory activity, wing display and pulse song between *D. melanogaster* and *D. simulans*, in circling and wing display between *D. elegans* and *D. gunungcola*, in pulse song among species of the *auraria* complex, between *D. serrata* and *D. birchii*, between *D. yakuba* and *D. santomea*, and between *D. pseudoobscura* and *D. persimilis*, and in timing of singing and song type between *D. affinis* and *D. algonquin* (Table 1). On the other hand, sexual signals are usually similar between allopatric close relatives: for example, the sine song frequency is similar between *D. lini* and *D. ogumai* (Wen et al., 2011).

4.1 Scissoring in *D. melanogaster* and *D. simulans*

Drosophila melanogaster and *D. simulans* are sympatric, cosmopolitan close relatives. Although they commonly meet and compete for food under natural conditions (Manning, 1960), they differ to some extent in their niches and behavior: *D. simulans* prefers lower temperatures than *D. melanogaster* does; *D. melanogaster* appears more active but *D. simulans* sluggish; and *D. melanogaster* pupates frequently on the walls of culture bottles but rarely on the food surface, while *D. simulans* does conversely. The commonest sequence of courtship is orientation-scissoring-vibration-licking in *D. simulans* and *D. melanogaster*, but scissoring is almost omitted from this sequence in *D. melanogaster* while *D. simulans* spends less time in wing vibration (Manning, 1960; Cobb et al., 1986). The amount of scissoring increased with courtship duration in *D. simulans* (Cobb et al., 1986). Given *D. simulans* males with *D. melanogaster* females, the *D. simulans* males act as *D. melanogaster* males, omitting scissoring after orientation. On the other hand, given *D. melanogaster* males with etherized *D. melanogaster* females, they display scissoring to the immobile females. These facts suggest that the repertoire of intrinsic courtship elements is common to both species (Manning, 1960). However, courtship is an interaction between two individuals, and changes in levels of excitation are necessary in both sexes. For more active females of *D. melanogaster*, visual stimuli by wing rowing and scissoring would be apt to be out of female's field of view and therefore ineffective, but wing vibration may have an effect in reducing female's locomotor activity (Cobb et al., 1986). Males of *D. simulans* may send more visual stimuli by scissoring their wings to excite conspecific females that are more sluggish, i.e., having a higher threshold for response, than *D. melanogaster* females (Manning, 1960). Interestingly, probably in relation to the shift of courtship to using more visual stimuli in *D. simulans*, the eyes of *D. simulans* are larger, with 5% more eye facets, than those of *D. melanogaster* (Manning, 1960).

4.2 Pulse song in the *D. pseudoobscura* species complex

Drosophila pseudoobscura and *D. persimilis* are sibling species with identical morphological characters and broadly sympatric across western North America. Females of these species discriminate against heterospecific males as mates, but males court heterospecific females as quickly and readily as they do conspecific females in the laboratory (Noor, 1996; Noor and Aquadro, 1998). Differences in the interpulse interval (IPI) and the intrapulse frequency (IPF) of courtship song have been detected between the two species. These differences are controlled by both X-chromosomal and autosomal loci (Noor and Aquadro, 1998).

4.3 Timing of singing and pulse song in the *D. quinaria* species group

Drosophila phalerata, *D. transversa*, *D. limbata* and *D. kuntzei* are fungal breeders and are distributed with overlap in parts of the United Kingdom and the near-continent. Several pulse song parameters are different between *D. transversa*, *D. limbata* and *D. kuntzei*: for example, the mean interpulse interval, IPI, is about 30 ms in *D. transversa*, 15 ms in *D. limbata*, but 13 ms in *D. kuntzei*. *Drosophila phalerata* is

very different in the timing and the type of courtship song from the other three species. It is the only species that sings during copulation and invests very little time in singing before mounting. It produces single pulses with no structural grouping but unimodal IPIs around the mean 370 ms. Each pulse is made up of 2 to 20 cycles and lasts 23 ms on average (Neems et al., 1997).

4.4 Female acceptability in the *D. mojavensis* species complex

Drosophila mojavensis and *D. arizonae* are sibling species and partially sympatric in northern Sinaloa, Sonora and in parts of southern Arizona. They utilize different rotting cacti species as primary substrates for breeding and occasionally meet together on the same host plant (Wasserman and Koepfer, 1977). Sympatric *D. mojavensis* females are much less receptive than its allopatric females to court by *D. arizonae* males, suggesting that selection on sympatric *D. mojavensis* has caused the range of female-acceptable male mating behaviors to move away from the range of *D. arizonae* behaviors (Wasserman and Koepfer, 1977; Markow, 1981a). However, *D. arizonae* males persist in courting *D. mojavensis* females under sympatry, even though they are unable to elicit an acceptance gesture (Markow, 1981a).

4.5 Courtship pattern in the *D. anceps* and the *D. eremophila* species complexes

Among three species of the *D. anceps* species complex (*D. anceps*, *D. leonis* and *D. nigrospiracula*), *D. nigrospiracula* is the first branched-off species and allopatric to the other two partially sympatric species. Courtship elements lost or gained in this complex are tapping and circling lost in *D. nigrospiracula*, and tapping and flicking lost in *D. leonis*; *D. anceps* show all these elements. The pulse burst length is significantly different between the sympatric species, *D. anceps* and *D. leonis*, but not between an allopatric pair of *D. nigrospiracula* and *D. anceps* (Alonso-Pimentel et al., 1995). On the other hand, three allopatric species, *D. eremophila*, *D. mettleri* and *D. micromettleri*, of the *D. eremophila* species complex belonging to the same *repleta* group have similar courtship patterns and courtship song parameters (Alonso-Pimentel et al., 1995).

5. Conclusion

Premating isolation may develop more rapidly than postmating isolation (Markow, 1981b). The basic similarity and the diversity of mating behavior displayed by various species of the Drosophilidae leave no doubt that there has been evolution of mating behavior in the family. Observing courtship behaviors of 101 *Drosophila* species, Spieth (1952) pointed out that tapping is an invariable element at the initial phase of mating sequence and therefore must have been present in the ancestral stocks. Most members of the subgenus *Drosophila* retain this ancestral contact feature of tapping and licking but produce no distance stimuli by wing display and vibration at the initial phase (Table 1), and therefore they are regarded as primarily contact animals. On the other hand, most members of the subgenus *Sophophora* have diverged from this ancestral pattern by becoming dependent on distance stimuli (wing display and vibration) rather than contact stimuli especially by licking (Table 1; Spieth, 1952). Of course, there are some exceptions in both subgenera, for example, female's duet song in the *virilis* group of the subgenus *Drosophila* (see Tomaru and Yamada, 2011 for a review on its features and roles) and licking in the *saltans* and the *willistoni* groups and the *melanogtaster* subgroup of the subgenus *Sophophora*. The precopulatory courtship usually accompanied with distance stimuli might be the derived courtship pattern, while the copulatory courtship widespread in insects and in the *montium* subgroup might be the primary type.

Behavioral differences may arise as by-products of genetic divergence in allopatric populations (Muller, 1939) or be developed and strengthened by natural selection in the process of reinforcement when allopatric populations secondarily become contact and their hybrids are poorly adapted (Dobzhansky, 1940). It has been documented by some selection experiments using *D. melanogaster* (McDonald, 1979) and *D. subobscura* (Pinsker and Doschek, 1980) that there is genetic variation in some traits of courtship behavior of *Drosophila*. Such intraspecific genetic variation should have been under natural selection and/or sexual selection and have been the main factor leading to evolution of courtship behavior.

Gain or loss of some courtship elements in ancestral or derived populations could result in unsuccessful courtship between the populations (Kaneshiro, 1976;

Watanabe and Kawanishi, 1979; Markow, 1981b). How courtship elements are gained or lost along evolutionary processes? Why such sexual signal divergence occurs more frequently between sympatric species than between allopatric ones? A possible answer will be provided on the basis of the reinforcement or reproductive character displacement models for speciation, although a number of theoretical problems have been pointed out on these models. Very rapid evolution of premating isolation observed between sympatric forms of *Drosophila* provides indirect evidence in favor of these models (Ritchie and Gleason, 1995). Under sympatry with closely related species, sexual selection alone may lead to increased divergence in their courtship behavior, and additionally, through severe resource competition, ecological selection could favor behavioral separation between them (Coyne and Orr, 1989; Kondrashov and Kondrashov, 1999; Ritchie, 2007). Thus, interactive forces from all environmental factors, such as food, habitat, and competing species, should be taken into account to fully understand mechanisms of courtship evolution. Population-based phylogeographic, ecological, genetic and genomic studies, and conducting experimental evolution are perhaps more likely to provide conclusive evidence in this genomic and proteomic era (Ritchie, 2007). We expect that further studies especially on more species in the *montium* subgroup shall give a deeper insight into the evolution of precopulatory and copulatory courtships in the *melanogaster* group.

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Structure and diversity of drosophilid communities in special relation to the three-dimensional structure of forest

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This paper reviews previous works on the vertical distributions of drosophilid flies in various forests ranging from the tropics to the subarctic region, especially along the Asian Green Belt, and addresses relationships between forest structure and drosophilid diversity on various spatial scales from the global to forest-stand ones, based on the data obtained by comparable, standardized collecting methods. The structure-diversity relationships show that on any spatial scales, more complex foliage structure contributes to the higher beta diversity along the vertical dimension and in consequence to higher species diversity in the whole community. Thus, the spatial habitat heterogeneity should be an important factor responsible for global to local variations in drosophilid species diversity. In forests with continuous, closed canopy, the vertical foliage structure defines the physical template of habitats, where inhabitant organisms actually interact with each other under various physical environments, and as a consequence determines the species diversity in inhabitant communities.

1. Introduction

Although there is much controversy for the magnitude of animal species richness on the earth (e.g., Gaston, 1991; Erwin, 1991), many researchers agree that a large part of animal diversity is due to species richness of arthropods, especially associated with the forest canopy in tropical rain forests (Erwin, 1983; Stork, 1988; Wilson, 1988). However, the forest canopy is still a less explored world on the earth. To reveal the great variety of life forms harbored there has been one of major challenges in ecology and biodiversity sciences.

In forest ecosystems, trees as skeleton organisms display leaves three-dimensionally in the aboveground space and construct complex structures in consequence of competition and niche separation for light. In general, the physical environments under the closed canopy are vertically heterogeneous but horizontally relatively homogeneous, resulting in a strato- or gradient-like pattern of physical environments. The stratified distribution of organisms living in such three-dimensional, vertically-structured forest spaces has been so widely documented as a ubiquitous pattern from boreal to tropical rain forests, in various kinds of organisms such as arthropods (Davidson, 1930; Fichter, 1939; Adams, 1941; Bates, 1944; Turnbull, 1960; Nielsen and Ejlersen, 1977; Sutton and Hudson, 1980; Sutton et al., 1983; Moeed and Meads, 1984; Brown et al., 1997; Davis et al., 1997), birds (Colquhoun and Morely, 1943; Stokes and Schultz, 1995), reptiles (Reagan, 1995), and mammals (Harrison, 1962; Francis, 1994; Whitmore, 1998; Bernard, 2001). The habitat heterogeneity hypothesis predicts a positive correlation between habitat complexity and species diversity: the greater the complexity of a habitat, the more the number of species that can co-exist in that habitat (Klopher and MacArthur, 1960; Pianka, 1966). This hypothesis has been tested to explain not only the latitudinal clines of species diversity on the global scale but also between-habitat

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differences on the local scale (see Tews et al., 2004, for review). Apparently, the ability for forests to harbor diverse animal species is not equivalent spatially on global (e.g., tropics vs. other regions), local (e.g., structurally complex vs. simple forests), and even microhabitat (e.g., the canopy vs. other strata) scales. This paper addresses various spatial patterns of diversity in a particular group of insects, drosophilid flies, which are strongly associated with forests in their lives and are commonly distributed across various kinds of forests from the tropics to the subarctic region.

With respect to the vertical distribution of drosophilid flies within forests, since a pioneer work by Basden (1953), only a few, fragmental reports have been published from Europe (Greuter, 1963; Shorrocks, 1975; Lumme et al., 1979), North America (Toda, 1985a), South America (Tidon-Sklorz and Sene, 1992) and Australia (van Klinken and Walter, 2001). In Asia, exceptionally, very intensive and extensive surveys on this subject have been conducted by the nearly identical method of sampling in various forests at many localities ranging from Southeast Asia to East Siberia (Toda, 1977b, 1984, 1985b, 1987, 1992; Beppu, 1980, 1984, 1985, 2006; Kwon and Toda, 1981; Toda and Fukuda, 1985; Toda et al., 1987; Tanabe et al., 2001; Tanabe, 2002; Ishii et al., 2004; Toda, unpublished data).

2. Spatial structure of drosophilid communities in cool-temperate forests

The vertical distributions of drosophilid flies were intensively studied in various cool-temperate forests of northern Japan, by setting specially-designed (for automatically retaining trapped flies into a bottle with fixative solution) traps, Toda's Trap II (Toda, 1977a) baited with fermented banana, vertically from the ground to the canopy. Toda (1977b) first recognized five patterns of vertical distribution among component species of a drosophilid community in a natural, broad-leaved forest, and divided the forest drosophilid community into two subcommunities, the understory and the canopy ones. The two subcommunities were different from each other in the taxonomic and the feeding-guild compositions of component species: the understory subcommunity was characterized by the *Drosophila quinaria* species group and composed mainly of herbage feeders and fungus feeders, while the canopy subcommunity included a number of species of steganine genera *Amiota* Loew, *Phortica* Schiner (cited as *Amiota* in Toda, 1977b) and *Leucophenga* Mik and of typical tree-sap feeders such as the *Drosophila obscura* species group. Toda (1987) further divided each subcommunity into two subassociations: the understory subcommunity into the herbage-layer subassociation and the shrub-layer one, and the canopy subcommunity into the lower subassociation distributed from the subarboreal to the lower canopy layer and the upper one restricted to the canopy layer. This vertically stratified structure of forest drosophilid community is relatively constant throughout the season (Toda, 1977b, 1987), and across natural forests at various localities (Toda, 1977b, 1984, 1987; Beppu, 1980; Toda, unpublished data), though being somewhat obscured in secondary forests with smaller aboveground space (Toda, 1987; Tanabe, 2002).

Toda (1987, 1992) investigated horizontal aspects in the three-dimensional structure of forest drosophilid community by covering the forest edge and/or gaps in study sites, and revealed that the living space of canopy subcommunity was remarkably lowered to the level of herbage layer at the forest edge but, on the other hand, that the understory subcommunity retreated interiorly from the forest edge. Moreover, Toda (1992) estimated, based on behavioral responses (i.e., the degree of vertical habitat segregation) of drosophilid flies to the vegetation structure, the subjective habitat patchiness or 'ecological neighbourhood' influential to the three-dimensional distribution of a forest drosophilid community to be on the scale of 110–450 m^2, corresponding well with the most prevalent size of canopy gaps occurring in various forests. Tanabe (2002) quantified the degree of stratification, i.e., the horizontal constancy of vertical distribution pattern, in each component species by observing their distributions at replicate plots within each study site of four forests with markedly different vertical foliage structures. Comparing the values among species and among forests, he found a tendency that feeding-specialist species depending on a particular food substrate, for example tree sap, showed highly predictable patterns of vertical stratification, while genelarist species showed non-stratified and/or highly variable patterns of vertical distribution.

As schematically shown in Fig. 1, the living space

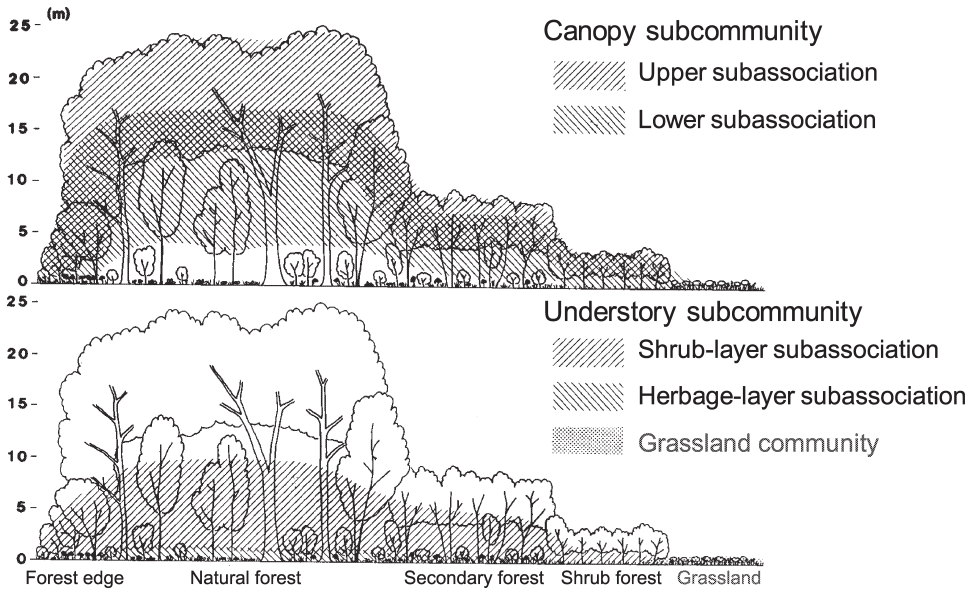

Figure 1 : Spatial structure of a drosophilid community in cool-temperate forests of northern Japan. [Reproduced with partial modification from Toda (1987) under the permission from Tokai University Press.]

of a forest drosophilid community is largely divided into two layers, the outer foliage layer (the canopy plus the edge) and the inner understory layer. This habitat stratification corresponds to the separation between production space and decomposition space in a forest ecosystem, probably reflecting the difference in food substances provided to drosophilid flies there. In addition to the primary separation between the underground and the aboveground forest ecosystems, this compartmentalization of the aboveground space should be fundamental for all organisms living there, as Richards (1983) argued, "More important than the stratification of the trees is the boundary between the euphotic zone in which the crowns are more or less fully exposed to sunlight (the 'canopy'), and the shaded oligophotic zone (the 'undergrowth') beneath."

3. Latitudinal cline

The gradient of species richness increasing from the poles to the equator is the most widely recognized pattern on the global scale. Such latitudinal gradients in species richness occur in diverse ecosystems such as open oceans, coasts, forests, deserts, rivers, lakes, etc. However, most of evidence for such clines is based on records of species in local fauna and/or flora across latitudes. If we want to evaluate the biodiversity, taking relative abundances of component species into account, across any transects, we need data taken quantitatively by standardized methods. There may be, however, very few such data for latitudinal clines of animal diversity. Nearly infinite numbers of surveys and samplings must have been conducted for animal diversity all over the world, but only few data may be comparable across localities because of differences in methods. Even slight differences in the design of sampling devices or in survey intensity bring about method-specific biases in samples and/or data. Here, latitudinal patterns of some biodiversity properties in drosophilid communities are presented, based on comparable data obtained by the completely standardized method across a wide range of latitudes from the equator to the subarctic region.

As reviewed in Section 2, drosophilid species segregate their microhabitats from each other in the aboveground space from the ground to the forest canopy. It is, then, expected that their biodiversity is much affected by the forest structure, especially the foliage stratification, which becomes simpler toward high latitudes. Fig. 2 shows results of a transect study on vertical distributions of drosophilid flies along the Asian Green Belt from a tropical rain forest in Sumatra to a boreal forest in East Siberia and even to a subarctic forest near the northern limit of timber line in the Mackenzie delta of Canada. Sampling procedures were standardized, setting the same banana traps (Toda's Trap II) vertically from the ground to the canopy (Toda and Kitching, 2002), although the number of traps varied among localities according to the heights of studied forests. The diversity of each local drosophilid community was evaluated by three measures, the species richness *S*,

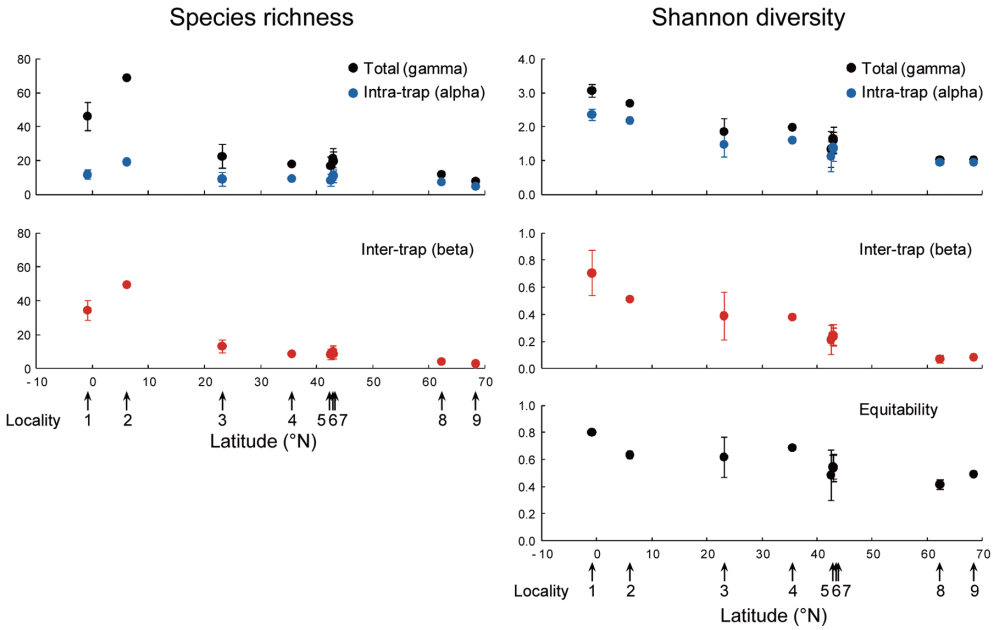

Figure 2 : Latitudinal clines of species richness, Shannon diversity (H') and equitability (Pielou's J') in forest drosophilid communities; for the first two measures, the total (gamma) diversity was partitioned into the intra-trap (alpha) and the inter-trap (beta) diversities. The mean and standard deviation (error bar) were calculated for weekly samples. Localities (latitude, the number of traps, the height of top trap, the number of weekly samples): 1) Padan, Sumatra, Indonesia (53' S, 10, 45 m, 3); 2) Poring, Sabah, Malaysia (6°5' N, 10, 40 m, 1); 3) Dinghushan, Guangdong, China (23°8' N, 7, 26 m, 35); 4) Ashiu, Kyoto Pref., Japan (35°30' N, 5, 15.7 m, 1); 5) Tomakomai, Hokkaido, Japan (42°30' N, 6, 13 m, 15); 6) Koryukozan, Hokkaido, Japan (42°51' N, 5, 16.5 m, 8); 7) Misumai, Hokkaido, Japan (42°57' N, 5, 14 m, 9); 8) Spaskayapad, Yakutia, Russia (62°14' N, 4, 10 m, 4), 9) Inuvik, Northwest Territories, Canada (68°22' N, 4, 10.5 m, 1).

the Shannon H' index and the equitability of Pielou's J':

$$H' = -\sum(n_i/N)\ln(n_i/N) \quad (1)$$

$$J' = H'/\ln S \quad (2)$$

where N is the total number of individuals, and n_i is the number of individuals of species i. In addition, for the first two measures, the total (gamma) diversity was partitioned into the intra-trap (alpha) and the inter-trap (beta) diversities along the vertical dimension of forest structure, according to the concept of "additive partitioning of species diversity" (Levins, 1968; Tanabe et al., 2001; Veech et al., 2002). Gamma diversity was based on summed data for all traps in each plot. Alpha diversity was estimated as the average of species richness or as the weighted (by the sample size) average of Shannon diversity calculated for each trap. Beta diversity caused by sample compositional differences among traps was calculated as the difference between the gamma and the alpha diversities (= gamma − alpha). The sampling frequency (sampling was repeated usually at weekly intervals) varied among localities, due to opportunity and conditions of field research: sampling was carried out nealy throughout the seasons at a few localities, but only once or twice at some localities. Therefore, the above diversity measures were presented as the means for weekly samples, with the standard deviations to show the seasonal variations in the cases of long-term sampling.

All the diversity measures, except for the intra-trap (alpha) species richness that was nearly constant across the latitudinal transect, decreased almost monotonically from the tropical forests to the boreal and subarctic ones. Thus, forest drosophilid communities showed a clear latitudinal cline, increasing southward, of the overall local (gamma) diversity. For the species richness, this cline (of gamma diversity) can be attributed mainly to the southward increasing vertical beta diversity, i.e., the rate of species turnover due to their micorhabitat segregation between strata from the understory to the canopy, which should be promoted by the larger (higher) and more finely stratified aboveground space in forests at lower latitudes. For the Shannon diversity, both alpha and beta diversities contributed to the southward increasing gamma diversity. And, the similarly southward increasing equitability indicates that relative abundances of component species are more balanced with each other in forest drosophilid communities at lower latitudes, probably reflecting the finer-grained niche space there.

4. Tree shape, forest structure and diversity of drosophilid community in boreal and temperate birch forests

Many hypotheses have been proposed to explain latitudinal clines of species diversity on the earth. Rohde (1992) reviewed a number of hypotheses and pointed out the logical circularity in many of them related to biotic factors, including 'biotic spatial heterogeneity' which this paper focuses on. He argued that those hypotheses are based on the assumption of higher diversity of some particular organisms, e.g., trees for the present case, in the tropics. Brown and Gibson (1983) claimed that ultimately, all general patterns of biodiversity must be attributed to physical causes. To explain the latitudinal cline of biodiversity, but only its northern part, in forest drosophilid communities from a bottom-up approach, Tanabe et al. (2001) compared tree shape, foliage structure and diversity of drosophilid community between two birch forests latitudinally about 20° apart from each other: one at Spaskayapad (62°14' N, 129°39' E) in East Siberian boreal region and the other at Nakagawa (44° 50' N, 142°15' E), Hokkaido in cool-temperate region.

Paltridge (1973) and Kuuluvainen (1992) showed theoretically the extreme importance of sun angle, which is determined by latitude, in shaping tree crowns. According to Kuuluvainen's (1992) simulations, latitudinally different light regimes are influential on the efficiency of photosynthesis in trees with different crown shapes; trees with planar crowns are more efficient at lower latitudes, while those with narrowly conical crowns are more efficient at higher latitudes. In reality, white birch (*Betula platyphylla* Sukatchev) trees had different crown shapes between the two surveyed forests; trees in the temperate forest had shallower and more planar crowns than those in the boreal forest (Fig. 3A). This indicates that the theory based on light regimes is applicable to the variation in crown shape of the same species, even within a small latitudinal range.

Based on the latitudinal cline of tree crown shape, Terborgh (1985) proposed a model to explain a latitudinal gradient in the vertical complexity of forest structure. According to his model, in tropical forests shallowly dome-shaped crowns permit sunlight to penetrate the canopy at shallow angles and enable the second foliage layer to develop close to the canopy. This process is potentially repeated, producing the third, fourth or more layers. However, in boreal forests, where trees with narrowly conical crowns are dominant, direct sunlight can pass through the canopy only at deep angles, so that establishment of a subcanopy layer is not possible. The observed difference in vertical complexity of foliage structure between the boreal and temperate birch forests supported Terborgh's (1985) theory. The temperate forest was more complex in the vertical foliage structure than the boreal forest; the former was characterized by the clearly two-layered foliage profile with the larger foliage height diversity (*FHD*) measured by the Shannon index, whereas the latter profile was less stratified, being distributed somewhat continuously from the ground to the canopy at lower densities and resulting in the smaller *FHD* (Fig. 3B). In addition, the horizontal connectivity of canopy foliage was significantly stronger, represented by a smaller proportion of gap, in the temperate forest than in the boreal one.

The difference in diversity between the two drosophilid communities in the temperate and boreal birch forests accorded with the trend of global latitudinal cline. As expected from the habitat heterogeneity hypothesis, the drosophilid community in the temperate forest was more diverse in all examined measures than that in the boreal forest (Fig. 4). The difference in species richness between the two communities was substantially attributed to the impoverishment of canopy-dwelling species in the boreal forest; the understory subcommunity had almost the same species richness in both forests. This must be related to the differences in foliage structure between the two forests. Fig. 5 shows schematically tree shapes, foliage structures and drosophilid communities in the two surveyed forests. As indicated by the lower horizontal connectivity of foliage, the canopy layer that consists of trees with deep, narrow crowns is highly rugged in the boreal forest. On the other hand, the canopy of the temperate forest, being formed by trees with shallow, somewhat spherical crowns due to more horizontal display of leaves, is more continuously waved. In addition, the boreal forest is vertically less stratified than the temperate forest. Thus, the depauperation of the canopy subcommunity in the boreal forest may have been caused by intensified competitive exclusion due to the reduction of living space or carrying capacity and the reduced degree of habitat

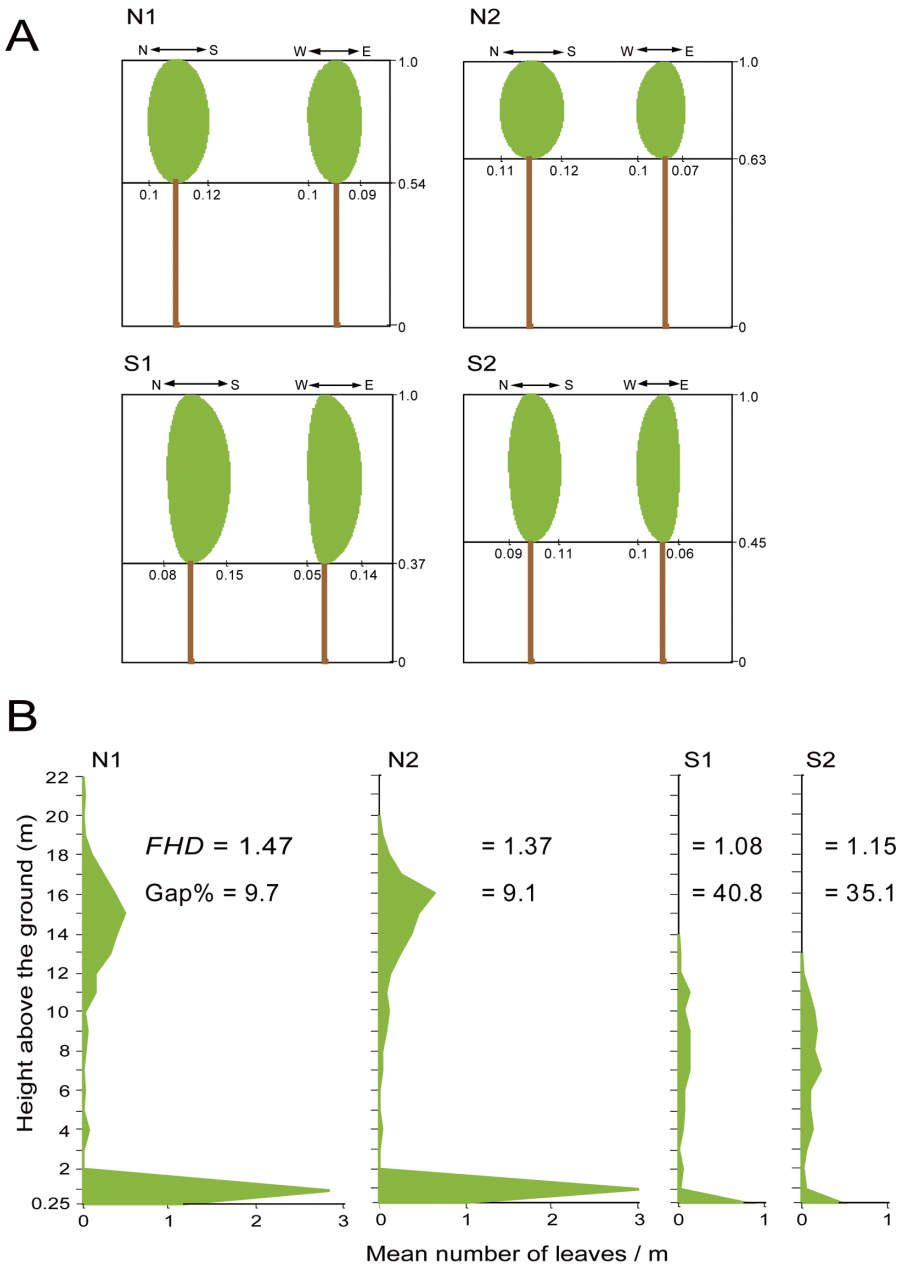

Figure 3：Average shape of white birch (*Betula platyphylla*) canopy trees (A) and average foliage height profile (B) in a cool-temperate forest (Nakagawa: N1, N2) and a boreal forest (Spaskayapad: S1, S2). In both forests, two study plots (10 m × 10 m quadrat) were established, near the center of which the traps were set vertically with a rope-pulley system. For all trees (≥ 1.5 m high) within each study plot, the following items were measured and/or recorded: species, the location on X-Y coordinates, tree height, trunk diameter at breast height (ca. 1.3 m high), height at the base of the lowest living branch, and crown radii in the four directions of cardinal points from the tree base. For comparisons of tree shape between localities and between plots, white birch that was common to both localities was selected in order to exclude the species-specificity in tree shape. In addition, the data for vertically erect canopy trees were used for calculation of the average shape to minimize the variation due to shading and leaning effects. To measure the vertical foliage distribution, MacArthur and Horn's (1969) camera method was employed (cf. Toda, 1992). The camera was equipped with a focusing screen with a grid of 16 intersections (focusing points) in the view field. The plot profile was obtained by averaging profiles at 36 intersections of 2 m mesh grid within each plot. Foliage height diversity (*FHD*) was measured by Shannon index, and the proportion of gap was calculated as the ratio of focusing points encountering the sky to the total points sampled within each plot. [Reproduced with partial modification from Tanabe et al. (2001) under the permission from Ecological Society of Japan.]

segregation.

The above results in combination support the habitat heterogeneity hypothesis, i.e., the positive correlation between habitat complexity and animal diversity, and suggest that a physical environmental factor, i.e., the sun angle, could be potentially important in producing latitudinal clines of diversity in animal communities inhabiting the aboveground space of forest through changes in plant structure at the individual and community levels.

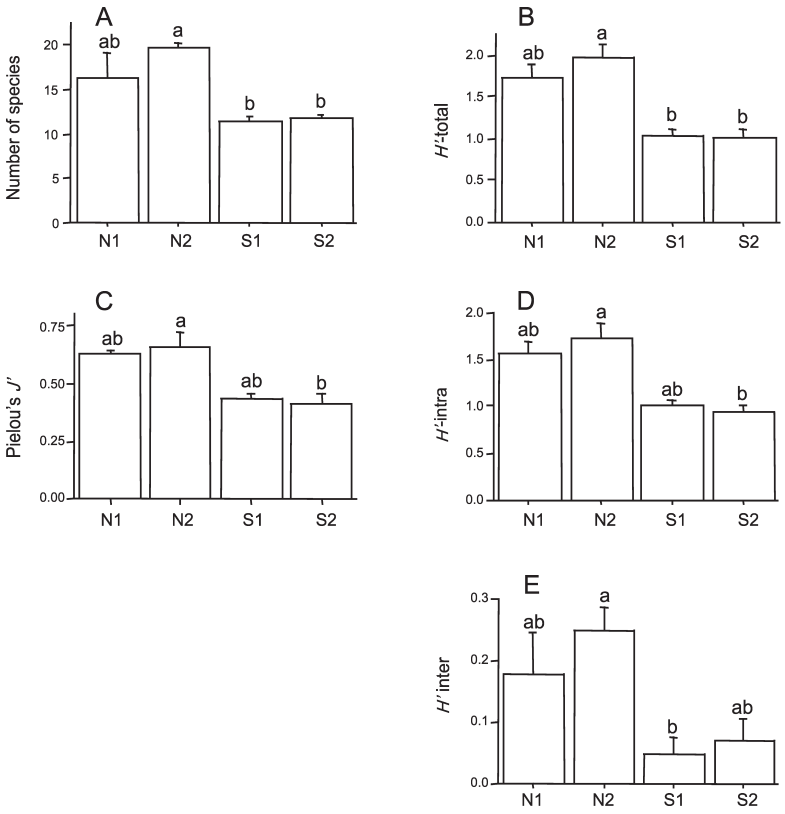

Figure 4：Means and standard deviations (error bars) of the number of species (A), the total (gamma) diversity measured by Shannon H' (B), the equitability of Pielou's J' (C), the intra-trap (alpha) diversity as the mean of H' for trap samples (D), and the inter-trap (beta) diversity as H'-total - H'-intra (E) at each plot in Nakagawa and Spaskayapad. The means and standard deviations were calculated for four weekly samples obtained at each study plot. The letters above the error bars indicate the results of Tukey-type multiple comparisons between the four plots; the same letters indicate non-significant differences ($P > 0.05$).

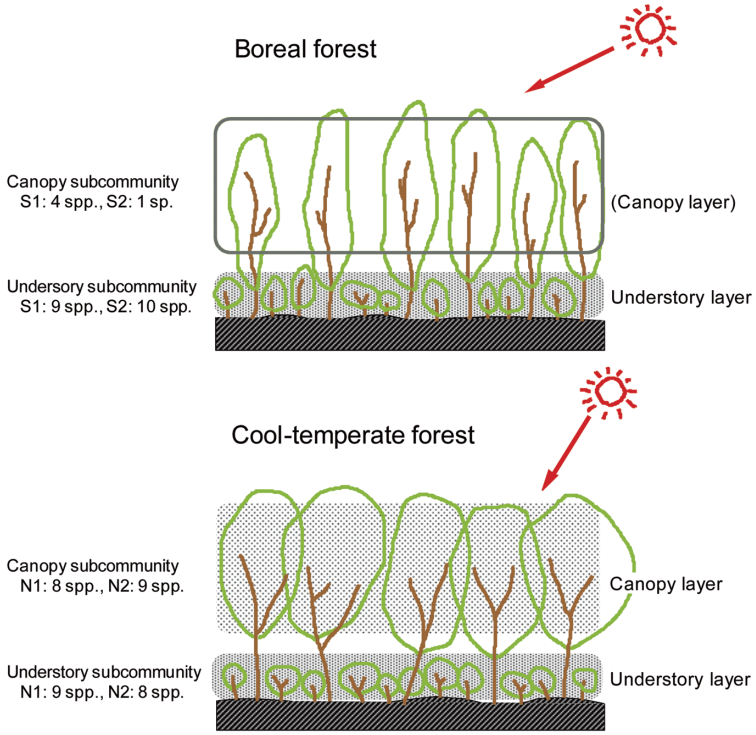

Figure 5：Schematic representation of sun angle, tree shape, forest structure and drosophilid community in boreal and cool-temperate birch forests, with indication of the numbers of species in canopy and understory subcommunities at each study plot.

5. Structure-diversity relationships on the local and forest-stand scales

Along the Asian Green Belt, the regional drosophilid species-pool, which should largely be a historical product of the past geological and biological processes, decreases in parallel with reducing complexity of forest structure from the tropics to the subarctic region; the Drosophilidae is considered to have originated and more diverged in the tropics (Throckmorton, 1975). Under such a latitudinal cline of regional species richness, nevertheless, Tanabe et al. (2001) showed that the forest structure was at least partly responsible for the depauperation of drosophilid canopy subcommunity in the boreal birch forest. In order to evaluate more accurately effects of the forest structure itself on the diversity of animals living there, it is necessary to compare animal communities among structurally different forests under the same regional species-pool. On a local scale, the forest structure changes both temporally according to vegetational succession (Aber, 1979) and spatially according to climatic and/or edaphic conditions (Aber et al., 1981; Terborgh, 1985). We present here some results of such a comparison among cool-temperate deciduous broad-leaved forests with different structural complexity.

Four structurally different forests (S1, S2, S3 and S4) were selected as study sites in the Tomakomai Experimental Forest (TOEF) of Hokkaido University, southern Hokkaido, Japan. The vertical foliage profiles were different among the four forests (Fig. 6), especially between the first two and the last two sites, and the average tree heights were 5–6 m, 11–12 m, 17–18 m, and 20–21 m, respectively, indicating a stepwise increment of each 4–5 m in forest height from S1 to S4. Four replicate survey plots were established in each site. The banana traps (Toda's Trap II) were set in the vertical arrangement at each plot and the trap heights (0.5, 1.5, 6.5, 11.5 and 16.5 m) were held almost constant over all sites (see Tanabe, 2002, for details of the study design).

Three habitat variables were measured at each plot: foliage height diversity (*FHD*), horizontal heterogeneity in vertical foliage distribution and the number of tree species. The second measure was calculated as the beta component of the foliage height diversity (*FHD*) among four subplots in a plot, according to the concept of additive partitioning of diversity. There were no correlations between these three variables across plots. Two diversity measures were calculated for the drosophilid community sampled at each plot: the total species richness excluding singletons as occasional migrants and the between-trap diversity in species composition. The latter was evaluated by Whittaker's (1960) *Bw*:

$$Bw = S/\alpha - 1 \quad (3)$$

where S is the total number of species, and α is the mean number of species per trap. These two diversity measures showed significantly positive correla-

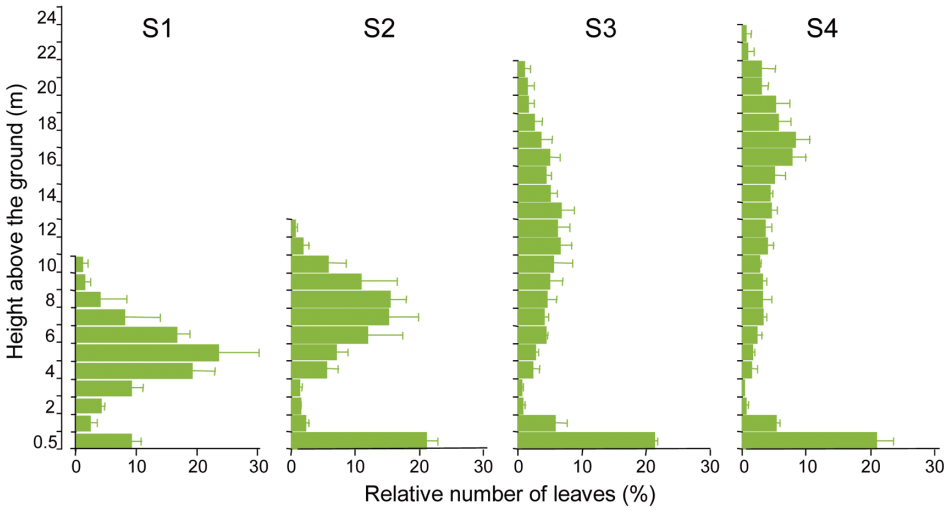

Figure 6 : Average vertical foliage profiles, with standard deviation of relative foliage density at each height (error bar), among four plots in four study sites, which were established in cool-temperate deciduous broad-leaved forests at different successional phases in the Tomakomai Experimental Forest of Hokkaido University, southern Hokkaido, Japan (42°40' N, 141°141' E).

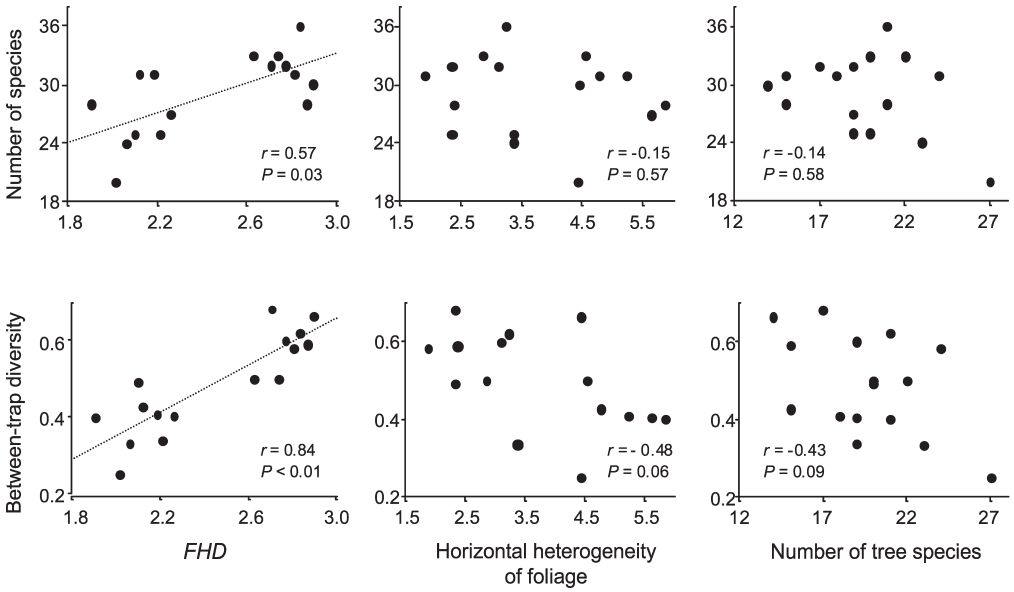

Figure 7 : Relations between drosophilid species diversity evaluated by two measures, species richness excluding singletons and between-trap diversity (Whittaker's *Bw*, see text) in species composition, and three habitat variables, foliage height diversity (*FHD*), horizontal heterogeneity of vertical foliage structure (the beta component of *FHD*, see text for details) and the number of tree species at 16 study plots (4 plots × 4 sites) in the Tomakomai Experiment Forest of Hokkaido University. Correlation coefficients (r) and P-values were based on Spearman's rank correlation tests.

tions only with *FHD* (Fig. 7). This is consistent with the patterns of drosophilid diversity on larger scales; i.e., the vertical foliage structure, *FHD*, influences the species diversity in forest drosophilid communities through limiting the degree of vertical microhabitat segregation among component species.

Furthermore, a number of studies have found vertical gradients of biodiversity in forests, with species-rich faunas at the canopy stratum (Erwin, 1983; Wilson and Peter, 1988; Stork, 1993). This within-forest pattern of variation in biodiversity is often independent of differences in stand-level structural properties. For example, in TOEF, Tanabe (2002) and Ishii et al. (2004) showed that the total number of drosophilid species collected at a stratum (trap height) increased from the ground to the canopy, and that this pattern was consistent among the forests differing markedly in vertical foliage structure. They suggested an important role of the unvarying nature of within-stand environmental gradients under the continuous, closed canopy in organizing forest drosophilid communities.

6. Implications for conservation of biodiversity and ecosystem functions in forests

The recent mass extinction of biological species (Chapin et al., 2000; Dirzo and Raven, 2003), caused by human activities, has resulted in serious deterioration of natural ecosystems. Ecosystems that lack their intrinsic complexity and functions cannot support diverse forms of life and will thus lead to further loss of biodiversity. This "degradation spiral", i.e., a positive feedback between loss of biodiversity and deterioration of ecosystem performances, will lead to the "crisis" of global ecosystems. The critical issue at present is to avoid triggering the "ecosystem degradation spiral". Biodiversity science, as a new multidisciplinary science, should contribute to developing a strategy for proper management of ecosystems, providing scientific data, predictions, and proposals for human societies to conserve biodiversity, its interactions and evolution in ecological complexity, and sustainable production by ecosystems of goods and services that meet human needs.

Summarizing this review, we emphasize that taking into account the three-dimensional structure of forest vegetation is essential for understanding the total amount and the uneven distribution of diversity in insect communities living there. As for drosophilid flies, it is a general pattern from boreal to tropical forests that the diversity of a local community is supported primarily by the vertical complexity of foliage stratification and secondarily by the horizontal variation in forest structure. If higher diversity of insects reflects and/or supports healthy functioning of forest ecosystem (it should be documented by fur-

ther studies), to maintain the complexity of three-dimensional forest structure is important for humankind to receive goods and services from forest ecosystems in a sustainable manner.

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低温科学 69 （2011） 113-119

小笠原諸島におけるショウジョウバエ相の変遷と新たな侵入種

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海洋島である小笠原諸島におけるこれまでのショウジョウバエ研究を概説するとともに，ショウジョウバエ相の変遷について議論した．また，1998 年 11 月に 1990 年の調査から 8 年ぶりに父島と母島で行なった調査の結果を報告した：(1)バナナトラップとスウィーピングにより，父島から 6 属 13 種 11,779 個体，母島から 7 属 14 種 15,178 個体のショウジョウバエが採集された；(2) *Drosophila hypocausta* が初めて小笠原諸島から記録され，*D. simulans* に次ぐ優占種（父島で総採集個体数の 19.4%，母島で 9.6%を占めた）であった．

Temporal changes of the drosophilid fauna in the Ogasawara Islands, with a report on a new colonizing species

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We reviewed previous researches on the drosophilid fauna of the Ogasawara Islands and discussed the recent 30-year changes of the drosophilid fauna in the islands. In addition, we reported the results of our collection by banana-trapping and net-sweeping in 1998 after a lapse of eight years from the latest survey; 11,779 individuals of 13 species of 6 genera were collected from Chichijima and 15,178 individuals of 14 species of 7 genera were collected from Hahajima. *Drosophila hypocausta* was collected from the Islands for the first time, and was the second most dominant species occupying 19.4% and 9.6% of the total samples in Chichijima and Hahajima, respectively.

1. はじめに

1.1 小笠原諸島と海洋島における生物研究

小笠原諸島は，父島列島，母島列島，聟島列島から構成されている小笠原群島に，西之島，火山列島，南鳥島，沖ノ鳥島を加えた約 30 の島々からなる．小笠原諸島と小笠原群島の呼称は行政的には区別されているが，一般的には混同されることもある．英語標記は，〝Ogasawara Islands〟，または無人島（ぶにんしま，ぶにんじま）という呼び名に由来する〝Bonin Islands〟である．近年の電子地図では「小笠原諸島」と〝Ogasawara Islands〟という標記が多い．

小笠原諸島の島々は一度も大陸と接したことのない海洋島であり，最も古い島は約 4 千万年前に形成された．小笠原諸島は形成時には，赤道付近に存在しており，その後北方に移動したとされている．海洋島としては，ハワイ諸島とガラパゴス諸島が有名であるが，ガラパゴス諸島は約 1 千万年前に，ハワイ諸島は最も古い島で約 520 万年前，一番新しい島はわずか 50 万年前に形成されたとされ（Bonacum et al., 2005），小笠原諸島と比べると比較的新しい．一方，小笠原諸島の島々は小さく，最も大きな父島と母島でそれぞれ 25 及び 24 km² であ

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るが，ハワイ諸島の島々は比較的大きく，最も新しい島であるハワイ島（10,433 km²）が最大であり，父島の約 400 倍ある．ガラパゴス諸島の最も大きな島は，イザベラ島（4,640 km²）であり，父島の 185 倍ある．

海洋島は大陸から遠くはなれて孤立していることから，生物相が特異的であり，進化と生態の観点から多くの研究がなされている．ガラパゴス諸島には，ダーウィンの進化論に大きな影響を与えたとされる多くの固有の生物が存在する．また，ハワイ諸島にも多くの固有生物が棲息するが，Hawaiian *Drosophila* と呼ばれる一群のショウジョウバエが適応放散の例として有名であり，教科書にも取り上げられている．ハワイ諸島のショウジョウバエについては，現在 559 種の固有種と 32 種の移入種が報告されているが，未記載種を含めると約 900 から 1,000 種の固有種が存在するといわれている（Leblanc et al., 2009; O'Grady et al., 2010）．ガラパゴス諸島ではダーウィンフィンチ等が有名であるが，ショウジョウバエは貧相であり，2 種の固有種と 16 種の移入種が報告されているのみである（Bächli, 2010）．小笠原諸島は，小さな島々からなるにも関わらず，特に母島では森林が発達していることもあり，固有種が 9 種と比較的多く，移入種も現在までに 18 種が報告されている（戸田・松長，1991）．種分化研究の対象地域として，小笠原諸島はハワイ諸島に遠く及ばないが，島々が小さいという点を生かして，生物の侵入，定着，消失という生態的プロセスの研究には適している．

1.2　小笠原諸島のショウジョウバエ研究史

小笠原諸島のショウジョウバエに関する最初の記録は，日本各地のショウジョウバエの記載と分布を報告した第 2 次大戦前の Kikkawa and Peng (1938) の論文に見ることができる．この論文では，小笠原諸島から *Drosophila melanogaster* Meigen, 1830（父島，母島，中硫黄島），*D. simulans* Sturtevant, 1919（父島，母島），*D. ananassae* Doleschall, 1858（母島，中硫黄島）の 3 種が報告されているが，個体数や生息状況には言及されていない．小笠原諸島は第 2 次大戦後しばらくアメリカに占領されていたが，この時代に Wheeler and Takada (1964) は小笠原を含むミクロネシアの島々のショウジョウバエを調査し，8 種のショウジョウバエを小笠原諸島から報告し，そのうちの 3 種，*Drosophila pectinifera* Wheeler & Takada, 1964, *Leucophenga boninensis* Wheeler & Takada, 1964, *Hirtodrosophila novicia* (Wheeler & Takada, 1964)（*Hirtodrosophila* は Grimaldi (1990) により属のレベルに格上げされた）を新種として記載している．*D. pectinifera* は小笠原諸島の固有種，*H. novicia* は小笠原諸島以外にパラウ島，*L. boninensis* はグァム島からも採集されている準固有種である．他の 5 種は，*D. melanogaster*，*D. ananassae*，*D. immigrans* Sturtevant, 1921, *D. annulipes* Duda, 1924, *Scaptomyza pallida* (Zetterstedt, 1847) であり，興味深いことに *D. simulans* の採集記録はない．1968 年に小笠原諸島が日本に返還された後は，まず Okada (1971, 1973) による分類・生態学的研究がある．Okada (1971) の研究は，1968 年から 1971 年にかけ複数の研究者による様々な方法で採集されたショウジョウバエ 9,450 個体に基づいており，16 種を報告している．新たに記録された種は，*Mycodrosophila gratiosa* (de Meijere, 1911)（小笠原産の種は Okada (1986) により，*Mycodrosophila serrata* として記載された），*Dettopsomyia nigrovittata* (Malloch, 1924), *D. suzukii* (Matsumura, 1931), *D. kikkawai* Burla, 1954, *D. daruma* Okada, 1956 と未記載の 4 種（*Nesiodrsophila* sp., *Scaptomyza* sp. I, *Scaptomyza* sp. II, *Hirtodrosophila* sp.）である．この調査では，これまでに報告されていた *H. novicia* と *D. ananassae* は採集されていない．最優占種は *D. immigrans* であり，*D. simulans*，*D. daruma*，*D. pectinifera* が続いている．*Drosophila daruma* は鳥の巣より多数採集されているが，それ以外では採集されていない．Okada (1973) では 4 種のショウジョウバエを記載している．1972 年に母島で採集された *Chymomyza fenestrata* Okada, 1973 と *Scaptomyza boninensis* Okada, 1973, *Scaptomyza hexasticha* Okada, 1973, *Hirtodrosophila apicohispida* (Okada, 1973) (= *Hirtodrosophila* sp., in Okada, 1971) である．*Scaptomyza hexasticha* に関しては，新亜属の *Boninoscaptomyza* が創設されている．

Toda (1976) は 1973 年 4 月に父島と母島で生息環境選好性，食性，繁殖場所，日周活動の調査を行ない，小笠原諸島のショウジョウバエ相の起源と群集構造を論議している．この調査では，父島で 4,027 個体，母島で 1,295 個体，両島合わせて 13 種のショウジョウバエが採集されており，新たに *D. busckii* Coquillett, 1901 を母島から報告している．また，森林環境では *D. pectinifera* と *L. boninensis* が優占しており，人家環境では *D. simulans* が最優占で，*D. immigrans*，*D. melanogaster* がそれに続いていた．

布山ほか（1980）は，小笠原諸島生物相の調査の一環として，1979 年 7 月と 12 月に父島と母島でショウジョウバエの採集を行なっている．この採集は主にバナナトラップを用いて人家環境でなされたこともあり，12 月の母島での採集以外では，森林性ショウジョウバエはほとんど採集されていない．この調査では，父島で 11 種 13,925 個体，母島で 13 種 9,609 個体，両島あわせて 15 種が採集された．また，新たに，*Scaptomyza elmoi* Takada, 1970 が 12 月に母島において 1 個体採集された．この論文では，小笠原産のショウジョウバエの食性と *D. simulans* と *D. melanogaster* の微視的分布に影響する環境要因についても考察している．さらに Fuyama

and Watada (1981) 及び Yamamoto et al. (1985) は，父島において *D. melanogaster* と *D. simulans* の微視的分布に関する研究を行なっている．前者は父島の清瀬地区で1,215個体の *D. melanogaster* と5,900個体の *D. simulans* を採集しており，後者は父島の清瀬と三日月山で合計2,058個体の *D. melanogaster* と13,834個体の *D. simulans* を採集している．Watada et al. (1986a, b) は日本本土と小笠原諸島の *D. melanogaster* と *D. simulans* のアロザイム変異と形態的形質を比較し，小笠原諸島の集団が遺伝的に異なっていることを明らかにしている．このことは，小笠原諸島の *D. melanogaster* と *D. simulans* が日本本土の個体群に由来しないことを示唆しており，小笠原諸島の最初の住民が欧米・ハワイ系住民であったことを考慮すると，この2種はこれらの人々の移住に伴って侵入した可能性がある．

Toda et al. (1987) は1979年から1985年にかけ，小笠原諸島での垂直分布の調査やスウィーピング，果物やキノコを用いたトラップ採集を行ない，2新種の記載，父島と母島における平衡種数とファウナの置換率推定，小笠原諸島と日本本土のショウジョウバエの群集構造の比較を行なった．新種として記載された種は，*Hirtodrosophila paiviae* (Toda & Rihimaa, 1987) と *Dichaetophora ogasawarensis* (Toda, 1987) (= *Nesiodrsophila* sp., in Okada, 1971) である（*Nesiodrosophila* Wheeler & Takada, 1964 は，Hu and Toda (2002) により，*Dichaetophora* Duda, 1940 の junior synonym とされた）．戸田・布山（1987）と戸田・松長（1991）は小笠原諸島におけるショウジョウバエ相の変遷をまとめ，父島と母島における平衡種数とファウナの置換率を推定している．戸田・松長（1991）は，1990年に父島（一部兄島）と母島で7属22種26,422個体のショウジョウバエを採集し，1990年における父島と母島の平衡種数をそれぞれ15種と20種，また，ファウナの1年あたりの置換率は，それぞれ2.05%と1.36%と推定している．この論文では，母島から新たに3種のショウジョウバエが報告されている．雄が1個体採集された *Drosophila immigrans* 種群 *hypocausta* 亜群に属する種は，後に *D. rubida* Mather, 1960 と同定された．他の2種は雌のみが採集されていて，1種は *Hirtodrosophila elliptosa* に近縁な新種であり，もう1種は *Mycodrosophila* 属の *Promycodrosophila* 亜属の新種であるが，まだ記載されていない．

2．1998年の採集結果

戸田・松長（1991）の調査（1990年11〜12月）から8年後の1998年11月3日から10日にかけ，小笠原諸島の父島と母島において，バナナトラップ，スウィーピングによる採集，及び落下果実からの採集を行なった．その結果，父島で6属13種11,779個体，母島で7属14種15,178個体，合わせて7属17種が採集された（表1）．特筆されることは，*Drosophila hypocausta* Osten-Sacken, 1882 が初記録され，かつ多数の個体が採集されたことである．*D. hypocausta* は，父島で2,286個体，母島で1,451個体採集され，父島における総採集個体数の19.4%，母島においても9.6%を占め，*D. simulans* に次ぐ優占種になっていた．一方，3番目に多い種は，父島では人家性種である *D. kikkawai* であったのに対し，母島では固有種で森林性の *D. pectinifera* であった．*Styloptera nishiharui* Okada, 1982 も父島で33個体が採集され，小笠原諸島からの初記録であった．また，過去に母島からのみ記録されていた *De. nigrovittata* が父島から初めて採集された．本種は，1980年以来母島から採集されておらず，小笠原諸島への再侵入である可能性がある．1958年以前に小笠原諸島から記録のあった *D. ananassae* はその後長い間記録がなく，1990年に母島への再侵入が確認されたが，今回は父島でのみ4個体が採集された．

表2は，地上に落下した果実上でのスウィーピングによって採集された成虫個体数と，それらの果実から羽化した個体数を示している．父島ではガジュマルの実（花嚢）から最も多くの成虫個体が採集され，グァヴァ，パパイヤ，タコノキと続いた．ガジュマルの実からは，*D. kikkawai*（675個体）と *D. simulans*（588個体）の成虫が多数採集され，*D. hypocausta* も112個体採集された．クワノキ，タコノキ，グァヴァからは *D. simulans* が独占的あるいは優占的に採集され，パパイヤでは *D. hypocausta* が，バナナの花では *D. kikkawai* が優占していた．一方，グァヴァからは *D. simulans* のみが羽化し，タコノキからは *D. simulans* と *D. melanogaster* が，ハスノハギリからは *D. kikkawai* と *D. hypocausta* が多数羽化した．母島ではヤエヤマアオキの実しか調査されなかったが，集まっていた成虫個体の多くは *D. simulans* と *D. pectinifera* であり，羽化個体のほとんどは *D. simulans* であった．この結果を，戸田・松長（1991）の調査結果と直接比較することは難しいが，共通していえることは，*D. simulans* と *D. pectinifera* の成虫がさまざまな果実で優占すること，*D. simulans* がそれらの果実で優占的に繁殖していることである．また，小笠原諸島に新たに侵入した *D. hypocausta* も比較的多くの果実を利用していることが明らかになった．

3．考察

3.1　小笠原諸島のショウジョウバエ相の変遷

1998年の調査結果を含めて，これまでに小笠原諸島から得られた全てのショウジョウバエの採集記録を，父

表 1：小笠原諸島（父島，母島）におけるショウジョウバエの採集記録

年(月)	−1958[a]		'68–72[b]		'73(IV)[c]		'79[d]		'80(XII)		'84(XII)		'85(XI)[e]		'90(XI-XII)		'98(XI)	
島	父	母	父	母	父	母	父	母	父	母	父	母	父	母	父	母	父	母
森林性種																		
Ch. fenestrata	(+)	(+)	(+)	5	10	2	3	1	?	(+)	?	(+)	−†	(+)	−	2	−	?
Sc. hexasticha	(+)	(+)	(+)	4	(+)	4	2	1	(+)	(+)	(+)	(+)	(+)	(+)	4	24	?	?
Di. ogasawarensis	(+)	(+)	(+)	2	1	(+)	882	7	(+)	(+)	(+)	(+)	7	(+)	138	784	4	3
H. paiviae	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	67	−	242	−	111
H. apicohispida	(+)	(+)	1	1	−†	−†	−	−	−	−	−	−	−	−	−	−	−	−
H. sp. aff. *elliptosa*	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	1	−	?
M. (*P.*) sp.	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	(+)	−	3	−	?
D. pectinifera	(+)	(+)	279	559	737	180	625	1129	941	(+)	+	(+)	616	344	798	1099	170	1025
St. nishiharui	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	33*	−
L. boninensis	+	+	154	265	13	142	161	290	(+)	(+)	(+)	(+)	3	(+)	323	180	53	29
H. novicia	−	+	−	(+)	−	(+)	−	1	−	(+)	−	(+)	−	(+)	−	4	−	30
M. (*M.*) *serrata*	?	?	53	22	14	1	4	19	4	(+)	(+)	(+)	1	5	39	104	?	51
D. daruma	?	−	1236	−	(+)	−	13	−	2	−	?	−	?	−	2	10*	?	26
D. annulipes	+	+	10	1	9	(+)	10	7	5	?	1	?	5	?	13	31	2	4
De. nigrovittata	−	?	−	79	−	(+)	−	5	−	−†	−	−	−	−	−	−	30*	−
草地性種																		
Sc. boninensis	(+)	(+)	1	4	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)	(+)	(+)	2	(+)	3	5
Sc. pallida	+	+	1	1	44	2	1	?	(+)	?	(+)	?	1	?	11	5	?	?
Sc. elmoi	−	−	−	−	−	−	−	1*	−	?	−	?	−	−†	45*	−	?	−
Sc. polygonia	−	−	−	−	−	−	−	−	?	−	?	−	1*	−	−†	−	−	−
準人家性種																		
D. suzukii	−	?	−	9	−	(+)	4*	107	2	(+)	1	(+)	27	38	197	138	4	338
D. kikkawai	?	−	44	−	2	−	644	182*	1192	(+)	+	(+)	11	1	29	49	1924	361
人家性種																		
D. simulans	+	+	2139	813	2722	418	11901	10215	18802	(+)	+	(+)	9324	12462	17355	4581	7179	11742
D. melanogaster	+	+	13	(+)	149	1	2664	606	2749	(+)	+	(+)	34	(+)	73	28	87	2
D. ananassae	+	+	−†	−†	−	−	−	−	−	−	−	−	−	−	−	1**	4**	?
D. immigrans	?	+	2077	1688	326	535	232	3	(+)	(+)	14	(+)	(+)	(+)	37	69	?	?
D. rubida	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1*	−	?
D. hypocausta	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	2286*	1451*
D. hydei	−	−	−	−	−	−	−	−	−	−	11*	−	−†	−	−	−	−	−
D. busckii	−	−	−	−	−	9*	2*	−†	−†	−	−	−	−	9**	−	−†	−	−
総個体数	?	?	6008	3442	4027	1295	17150	12574	23695	−	?	−	10030	12926	19066	7356	11779	15178
採集された種数	6	8	12	14	11	10	15	15	7	−	8	−	11	7	15	20	13	14
推定総種数	?	?	15	19	14	19	16	19–20	14–16	16–19	14–17	16–19	14–15	17–19	15	21	13–19	14–22

(+)サンプリングエラー，*新記録，**再侵入，†絶滅が推定されるケース．

出典：[a]Kikkawa and Peng (1938), Wheeler and Takada (1961); [b]Okada (1971, 1973); [c]Toda (1976); [d]一部，布山ら(1980); [e]Toda et al. (1987).

表 2：地上に落下した果実のまわりで捕虫網によって採集された成虫個体数といろいろな果実からの羽化個体数

島	父島								母島		総計
果実	クワ	タコノキ	ガジュマル	グァヴァ	ハスノハギリ	パパイヤ	バナナの花	計	ヤエヤマアオキ		
成虫個体数											
D. pectinifera	−	−	27	11	11	2	−	51	24		75
L. boninensis	−	−	−	1	−	−	−	1	−		1
Sc. boninensis	−	−	−	−	−	1	−	1	−		1
D. suzukii	−	−	−	−	−	1	−	1	2		3
D. kikkawai	−	−	674	3	3	−	53	733	17		750
D. simulans	127	186	588	260	6	−	−	1167	297		1464
D. melanogaster	−	1	2	1	−	−	−	4	−		4
D. ananassae	−	−	1	−	−	−	−	1	−		1
D. hypocausta	−	−	112	14	1	211	3	341	27		368
計	127	187	1404	290	21	215	56	2300	367		2667
羽化個体数											
D. pectinifera	−	−	−	−	1	−	−	1	−		1
D. kikkawai	−	−	4	−	35	−	−	39	−		39
D. simulans	−	42	2	79	3	−	−	126	190		316
D. melanogaster	−	27	−	−	−	−	−	27	−		27
D. hypocausta	−	2	2	−	22	−	−	26	1		27
計	−	71	8	79	61	−	−	219	191		410

島と母島に分け，表1に示した．また，戸田・松長（1991）の方式に従って，サンプリングエラー，絶滅，移入を推定し，父島と母島それぞれにおける総種数を算定した．1998年の推定値は，父島，母島においてそれぞれ13–19，14–22とかなりの幅があるが，1968–’70年から1990年までの推定総種数も，それぞれ14–17，16–21であり，ともに比較的安定しているといえそうである．

しかし，MacArthur and Wilson (1967) が動的平衡モデルにより予測しているように，その構成種の置換はかなり頻繁に起きている．特に，かつて優占種の1種であった *D. immigrans* は近年その個体数を急激に減少させた．1968–’72年の記録によると，*D. immigrans* は母島で最優占種（相対頻度49%）であり，父島でも第1位の *D. simulans*（36%）に匹敵する優占種（35%）であったが，1973年には父島で優占度が8%に落ち，1979年に母島で激減，その後両島でほとんど採集されなくなった（1990年には，父島，母島でそれぞれ37個体，69個体が採集されている）．1998年の採集では，*D. immigrans* は両島から全く記録されず，興味深いことに，それと置き換わるように同じ *immigrans* 種群に属する *D. hypocausta* が初記録され，なおかつ父島，母島それぞれで19.4%，9.6%を占め，*D. simulans* に次ぐ第2優占種になっていた．*D. hypocausta* が属する *hypocausta* 亜群のショウジョウバエは，東南アジアを中心に，ニューギニア，オーストラリア，ミクロネシア，琉球列島に分布しており（Hihara and Lin, 1984），小笠原諸島は琉球列島と並んでこのグループの分布圏の北限に位置する．過去40年間で，父島の気温は約0.7度上昇しており，熱帯性の *hypocausta* 亜群の種の小笠原諸島への侵入と，暖温帯性の *D. immigrans* の減少（絶滅）は，その直接の影響かもしれない．あるいは，*D. immigrans* の衰退の原因は，戸田・松長（1991）が指摘しているように *D. simulans* や新たに侵入してきた *D. hypocausta* との競争に，温暖化が間接的に影響した結果かもしれない．いずれにしても，今後の小笠原諸島における推移と，似た環境条件下にある琉球列島などでの並行した調査が必要である．

3.2　群集組成の変化

構成種の相対頻度を加味した群集組成の変化を見るために，父島の三日月山の二次林，大村の人家周辺，母島の乳房山山麓の自然林，沖村の人家周辺でのショウジョウバエ群集の多様度の変化を表3に示した．多様度の指標としては，種数，平均多様度（Shannonの *H’*），均等度（Pielouの *J’*）を，最優占種である *D. simulans* の相対頻度と共に示している．この表から明らかなように，初期（1973–’79年）は *D. simulans* の相対頻度がそれほど高くなく，群集の多様度も比較的高かったが，その後1990年まで *D. simulans* の独占状態が継続し，群集組成が単純化してきている．1990年の採集では，*D. simulans* の相対頻度は調査した4カ所で90–97%にもなっている．しかし，1998年には，*D. hypocausta* が侵

表3：果物トラップで採集されたサンプルの多様度と *D. simulans* の優占度の変化

年	1973	1979	1979	1979	1985	1990	1998
月	IV	VII	XI	XII	XI	XI–XII	XI
父島，三日月山二次林							
総個体数	80	−	−	351	9612	11244	1358
総種数	5	−	−	3	7	7	4
多様度：*H’*[1]	1.022	−	−	0.267	0.222	0.158	0.737
均等度：*J’*[2]	0.635	−	−	0.243	0.114	0.081	0.531
D. simulans の相対頻度（%）	60.0	−	−	94.0	95.2	97.1	62.4
父島，大村人家周辺							
総個体数	1158	641	−	1146	−	4267	719
総種数	6	6	−	3	−	6	7
多様度：*H’*	0.826	1.447	−	0.473	−	0.187	1.075
均等度：*J’*	0.461	0.808	−	0.431	−	0.104	0.552
D. simulans の相対頻度（%）	74.5	35.7	−	82.3	−	96.7	58.8
母島，乳房山山麓自然林							
総個体数	−	−	160	2126	6274	188	14061
総種数	−	−	3	4	3	5	7
多様度：*H’*	−	−	0.686	0.141	0.179	0.402	0.745
均等度：*J’*	−	−	0.624	0.102	0.163	0.250	0.383
D. simulans の相対頻度（%）	−	−	63.8	97.6	96.0	91.5	80.0
母島，沖村人家周辺							
総個体数	−	203	−	2480	−	1075	−
総種数	−	4	−	8	−	7	−
多様度：*H’*	−	0.773	−	0.718	−	0.494	−
均等度：*J’*	−	0.558	−	0.345	−	0.254	−
D. simulans の相対頻度（%）	−	36.5	−	80.3	−	89.9	−

[1] $H' = -\Sigma P_i \ln P_i$，[2] $J' = H'/\ln S$，ただし，P_i は種 i の比率，S は総種数．

入，増加した影響で，調査した 3 カ所すべてで *D. simulans* の相対頻度が 59–80％と減少し，平均多様度と均等度が上昇した．

小笠原諸島のショウジョウバエ群集において，1979 年以降一貫して *D. simulans* が最優占種であったが，その相対頻度は他の群集構成種との関係で多少とも変動してきた．1979 年と 1980 年の採集では，近縁種の *D. melanogaster* が父島で 2,000 個体以上，母島でも 500 個体以上が採集されている．この *D. melanogaster* の増大した時期は，小笠原諸島においてミカンコミバエの駆除が行なわれていた 1975 年から，根絶事業が終わった 1985 年までの時期と一致する．小笠原支庁によるミカンコミバエの駆除事業では，父島に害虫研究室が設置され，多量の不妊ミカンコミバエを生産するために多くの「ふすま」が使用・放置されており，この「ふすま」が *D. melanogaster* の発生源であった可能性が高い．実際，1985 年に害虫研究室がなくなった後は，1998 年にいたるまで *D. melanogaster* の採集個体数は激減した．一方，小笠原諸島の固有種で，唯一個体数が多いのが *D. pectinifera* である．*D. pectinifera* の相対頻度は，数％から 20％でばらついているが，長期間の増減に関しては特定の傾向は見られない．しかし，1998 年の採集では，父島で 1.4％，母島で 6.8％となり，父島での相対頻度はこれまでで最低である．

海洋島の固有生物相に影響する要因としては，生息環境の変化が最も重大であるが，外来種の侵入も無視できない（Hardy, 1974）．小笠原諸島の固有種である *D. pectinifera* の相対頻度は，*D. simulans*（1998 年は 61％）や *D. hypocausta*（19％）と比較すると非常に低い．移入種である *D. simulans* や *D. hypocausta* は，小笠原諸島では自然林でも優占種となっている．森林性種で幅の広い食性を持つ *D. pectinifera* の個体群動態の追跡が，固有種の保護の観点からも非常に重要である．*D. hypocausta* の侵入・定着は，小笠原諸島のショウジョウバエ相の変遷に，新たな展開を引きおこす可能性があり，小笠原諸島におけるショウジョウバエの侵入，定着，消失という生態的プロセスの解明には，今後の継続調査が非常に重要である．また，これまで小笠原諸島のショウジョウバエ相は主に晩秋から冬季にかけて調査されることが多かったが，今後は春期や夏期に調査を行なうことも，ショウジョウバエ相の変遷の全貌を明らかにするうえで必要であろう．

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Faunal and seasonal surveys on drosophilid flies by net sweeping in Ehime Prefecture, Shikoku, Japan

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A faunal survey on drosophilid flies by net sweeping was carried out in Ehime Prefecture, Shikoku, Japan from October 2009 to October 2010. A total of 11,663 individuals of 54 species belonging to 15 genera were collected by the year-round samplings at two localities, Dogo Park and Mt. Miyukiji, and occasional ones at another site in Matsuyama. The most abundant species was *Liodrosophila aerea* (49%), followed by *Drosophila rufa* (17%), *D. lutescens* (9.6%), *D. sternopleuralis* (4.0%) and *D. bizonata* (3.8%). The six dominant species showed more or less similar patterns in seasonal population fluctuation, being abundant in spring and/or fall with some variation between the localities. The observed seasonal patterns of drosophilid flies are discussed in relation to the extraordinary hot weather in the summer of 2010.

1. Introduction

Although the drosophilid fauna of the Japan Archipelago is better known (in total 307 species; Toda, 2006-2010) in comparison to other regions of the world, intensively explored areas are still limited. In Hokkaido, Momma, Toda and their collaborators have clarified the basic information on its drosophilid fauna and seasonality through the serial papers of "*Drosophila* survey of Hokkaido" and "Bionomics of Drosophilidae (Diptera) in Hokkaido". In addition, continuous ecological studies of drosophilid species in Hokkaido have been carried out on daily activity, migration, food preference, diapause, etc. (Toda, 1973; Kimura, 1988; Kimura and Toda, 1989; Kimura and Beppu, 1993). Toda (1984) compared guild structure between drosophilid communities in Morioka, Iwate Prefecture and Kiyosumi, Chiba Prefecture. In the central part of Japan, faunal and ecological surveys were carried out in Shiga Height, Nagano Prefecture and Imperial Palace grounds, Tokyo (Beppu, 2000, 2001, 2006). Drosophilid faunas of subtropical islands were relatively intensively surveyed on the Ogasawara Islands (Watada et al., 2011), and Iriomote-jima and Kume-jima of the Ryukyu Islands (Hirai et al., 2000; Kondo and Kimura, 2008).

On the other hand, only few fragmentary information has been brought from the western mainland of Japan on its drosophilid fauna. Watada et al. (2000) revealed the predominance of two sibling species, *Drosophila simulans* and *D. albomicans*, in domestic and semi-domestic areas in Ehime Prefecture, using banana-bait traps; *D. albomicans* that was originally a tropical and subtropical species was dominant from the summer to the fall in the urban area in Matsuyama, while *D. simulans* was the most abundant from October to December. By banana trap collections, Hoshina and Watada (2001) studied distribution of drosophilid flies in natural forests and Japanese cedar forests at several localities in Ehime, and showed the predominance of *D. immigrans* in both types of the forests. Along with these results, the two previous studies by banana-bait trapping, in combination, brought faunal records of 28 species belonging to three genera (*Drosophila*, *Scaptodrosophila* and *Styloptera*) from Ehime Prefecture. It is, however, very difficult to reveal the entire fauna of drosophilid flies by banana trap collection, because a number of drosophilid species are not attracted to banana-bait traps. In the present study, we collected drosophilid flies by net sweeping in a semi-natural park and along

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2. Collection localities and methods

Drosophilid flies were collected from October 2009 to October 2010 in Matsuyama (33°N, 132°E), by net sweeping at two localities (Dogo Park and Mt. Miyukiji) in Matsuyama, Ehime Prefecture. The two localities are only 1.5 km apart from each other. Dogo Park is a semi-natural woods with a small hill (71 m in altitude) located in an urban area, and Mt. Miyukiji is a small mountain (165 m in altitude), both being close to a small mountain range. Sweeping was made with an insect net along trails of nearly the same length (700 m) at both localities. In addition, three occasional collections were made at the third locality, Matsuyama General Park, with many planted camellia trees but less weeds. Collections were usually made four times a month at Dogo Park and Mt. Miyukiji, but more frequently, eight to 15 times, in each of several early months of the collection period (Tables 1 and 2). To compare seasonal changes in

Table 1 : Number of drosophilid flies monthly collected at Dogo Park

Year	2009			2010										
Drosophilid species/Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	**Total**
Steganinae														
Portica magna	3	–	–	–	–	1	1	–	–	–	–	–	–	**5**
P. okadai	6	–	–	–	–	1	5	–	1	–	–	–	–	**13**
Leucophenga angusta	3	1	–	–	–	–	–	–	1	–	–	4	1	**10**
L. bellula	2	–	–	–	–	–	–	–	–	–	–	–	–	**2**
L. concilia	–	–	1	–	–	–	–	–	–	–	–	–	–	**1**
L. interrupta	10	1	–	–	–	1	–	3	4	1	1	–	1	**22**
L. maculata	3	1	–	–	–	1	–	1	–	–	–	–	–	**6**
L. orientalis	4	1	–	–	–	7	–	–	–	–	–	–	–	**12**
L. ornata	1	–	–	–	–	–	–	1	–	–	–	–	–	**2**
Drosophilinae														
Microdrosophila (*Mi.*) *maculata*	–	1	–	–	–	1	–	–	–	–	–	–	–	**2**
Mi. (*Mi.*) *pseudopleurolineata*	1	11	6	3	2	2	31	2	–	3	1	1	1	**64**
Mi. (*Oxystyloptera*) *urashimae*	–	–	–	1	–	2	33	4	–	–	–	–	–	**40**
Liodrosophila aerea	193	1110	561	153	371	895	641	616	147	37	–	53	276	**5053**
Li. castanea	–	–	–	–	–	–	–	–	–	1	–	–	–	**1**
Dichaetophora acutissima	–	27	18	9	34	67	33	2	2	–	–	1	–	**193**
Mycodrosophila gratiosa	2	3	1	–	1	–	1	1	1	1	–	1	–	**12**
Paramycodrosophila nakamurai	–	–	–	–	–	–	2	3	2	–	–	–	–	**7**
Collessia kirishimana	–	–	–	–	–	–	–	–	–	4	–	–	–	**4**
Scaptomyza (*Parascaptomyza*) *pallida*	2	1	2	–	–	–	15	38	–	–	–	–	–	**58**
Sc. (*Scaptomyza*) *graminum*	3	1	–	1	–	3	2	7	–	–	–	–	–	**17**
Zaprionus (*Aprionus*) *aungsani*	1	–	–	–	–	–	–	–	–	–	–	–	–	**1**
Scaptodrosophila coracina	–	–	–	–	–	25	15	5	–	–	–	–	1	**46**
Sa. subtilis	–	–	–	–	–	10	7	–	–	–	–	–	–	**17**
Sa. sp.	5	11	2	–	–	–	–	–	–	1	1	–	1	**21**
Hirtodrosophila mediohispida	–	–	–	–	–	1	–	–	–	–	–	–	–	**1**
Drosophila (*Sophophora*) *tukubaensis*	–	–	–	–	–	1	3	–	–	–	–	–	–	**4**
D. (*S.*) *oshimai*	–	1	–	–	10	70	3	–	–	–	–	–	–	**84**
D. (*S.*) *subpulchrella*	–	2	1	–	–	2	1	–	–	–	–	–	–	**6**
D. (*S.*) *suzukii*	11	14	8	8	4	36	16	–	1	–	–	–	–	**98**
D. (*S.*) *lutescens*	7	23	29	15	35	156	525	155	55	–	–	–	2	**1002**
D. (*S.*) *simulans*	1	–	–	–	–	–	–	–	–	–	–	–	–	**1**
D. (*S.*) *ficusphila*	16	15	3	–	1	3	–	–	–	–	–	–	1	**39**
D. (*S.*) *auraria*	–	–	–	–	1	9	7	6	47	12	1	–	–	**83**
D. (*S.*) *biauraria*	–	–	–	–	–	1	1	–	–	–	–	–	–	**2**
D. (*S.*) *rufa*	89	273	93	31	103	211	167	273	87	150	–	3	38	**1518**
D. (*Drosophila*) *hydei*	–	–	–	–	–	2	–	1	–	–	–	–	–	**3**
D. (*D.*) *brachynephros*	6	7	–	–	–	1	1	–	–	1	–	–	–	**16**
D. (*D.*) *nigromaculata*	–	–	–	–	–	–	–	1	–	–	–	–	–	**1**
D. (*D.*) *bizonata*	85	59	14	3	6	16	8	7	6	21	1	13	12	**251**
D. (*D.*) *sternopleuralis*	7	26	5	2	6	221	99	27	10	1	–	5	29	**438**
D. (*D.*) *albomicans*	12	38	6	–	–	–	–	–	–	2	–	–	2	**60**
D. (*D.*) *annulipes*	–	2	1	2	–	–	–	–	–	–	–	1	–	**6**
D. (*D.*) *curviceps*	–	1	3	2	2	85	37	8	2	–	–	–	–	**140**
D. (*D.*) *immigrans*	11	16	4	2	–	10	38	103	34	1	–	–	–	**219**
D. (*Dorsilopha*) *busckii*	–	–	–	–	–	1	1	5	1	1	–	–	–	**9**
Total number of individuals	484	1646	758	232	576	1842	1693	1269	401	237	5	82	365	**9590**
Number of species	25	23	16	13	13	30	26	22	16	15	5	9	12	**45**
Number of collections	13	15	8	5	2	8	4	4	4	4	4	4	4	

population density among six dominant species, the numbers of their collected individuals were standardized by four times of collection per month (Figs 1 and 2). Most of collected flies were transferred into 70% alcohol and classified under a dissection microscope. Females of *D. lutescens* and *D. takahashii* that were morphologically indistinguishable were individually cultured, and males of their F1 progenies were used for species identification. It was impossible to identify females of *Amiota* species that could be neither distinguished morphologically nor bred in laboratory. Then, females of *Amiota* species were pooled as *Amiota* spp. in Table 2.

3. Results and discussion

3.1 Drosophilid fauna in Ehime

A total of 11,590 individuals of 53 species belonging to 15 genera in 2 subfamilies (9,590 individuals, 45 species, 13 genera at Dogo Park, and 2,000 individuals, 43 species, 14 genera on Mt. Miyukiji) were collected in the present study (Tables 1–3). As for the genus *Amiota*, all individuals of this genus were collected at the same point of Mt. Miyukiji and included males of

Table 2 : Number of drosophilid flies monthly collected on Mt. Miyukiji

Year	2009			2010										
Drosophilid species/Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	**Total**
Steganinae														
Stegana (*Steganina*) *nigrifrons*	–	–	1	–	–	–	–	–	–	–	–	–	–	**1**
Amiota furcata	–	–	–	–	–	–	–	–	1	1	–	–	–	**2**
A. spp. ♀	–	–	–	–	–	–	–	1	1	1	–	–	–	**3**
Phortica magna	1	–	–	–	1	6	2	1	2	1	–	–	–	**14**
P. okadai	8	1	–	–	–	1	–	–	2	15	3	1	–	**31**
P. sp. aff. *glabra*	–	–	–	–	–	–	–	–	–	–	–	–	1	**1**
Leucophenga angusta	3	2	–	–	–	–	–	2	1	–	–	15	1	**24**
L. bellula	2	–	1	–	–	–	–	–	–	–	–	–	–	**3**
L. interrupta	23	2	–	–	–	–	–	–	–	4	–	–	–	**29**
L. maculata	–	2	–	–	–	–	–	1	1	–	1	–	–	**5**
L. orientalis	4	–	–	–	–	–	1	–	–	–	–	–	1	**6**
L. ornata	–	–	–	–	–	–	–	–	1	1	–	–	–	**2**
Drosophilinae														
Microdrosophila (*Mi.*) *pseudopleurolineata*	2	2	–	–	–	–	–	–	–	–	–	2	1	**7**
Liodrosophila aerea	34	124	163	33	56	10	3	68	10	25	–	21	74	**621**
Dichaetophora acutissima	–	2	9	1	1	–	1	–	–	–	–	–	–	**14**
Di. delicata	1	–	–	–	–	–	–	–	–	–	–	–	–	**1**
Mycodrosophila gratiosa	–	–	1	–	–	–	–	–	3	76	–	–	1	**81**
My. planipalpis	–	–	–	–	–	–	–	1	1	–	–	–	–	**2**
My. poecilogastra	–	–	–	–	–	–	–	1	–	23	–	–	–	**24**
Paramycodrosophila nakamurai	–	–	–	–	–	–	–	2	1	2	–	–	–	**5**
Collessia kirishimana	–	–	–	–	–	–	–	–	–	2	–	–	–	**2**
Scaptomyza (*Parascaptomyza*) *pallida*	1	–	4	8	–	–	5	2	–	–	–	–	–	**20**
Sc. (*Scaptomyza*) *graminum*	1	–	1	–	–	–	–	–	–	–	–	–	–	**2**
Scaptodrosophila coracina	–	1	–	–	–	–	–	–	1	–	–	–	–	**2**
Sa. subtilis	–	2	–	–	–	–	–	–	–	–	1	–	–	**3**
Sa. sp.	9	1	3	–	–	–	–	–	–	7	–	–	–	**20**
Hirtodrosophila mediohispida	–	–	1	–	–	–	–	–	–	–	–	–	–	**1**
Drosophila (*Sophophora*) *oshimai*	–	–	–	–	–	–	–	1	–	–	–	–	–	**1**
D. (*S.*) *subpulchrella*	–	–	2	–	–	1	–	–	–	–	–	–	–	**3**
D. (*S.*) *suzukii*	19	17	34	3	7	–	1	–	–	–	–	–	–	**81**
D. (*S.*) *lutescens*	7	21	29	–	1	–	1	31	13	3	–	–	2	**108**
D. (*S.*) *takahashii*	–	–	–	–	–	–	–	–	–	3	1	–	–	**4**
D. (*S.*) *melanogaster*	1	–	–	–	–	–	–	–	–	–	–	–	–	**1**
D. (*S.*) *ficusphila*	9	3	8	1	–	–	–	–	–	–	–	–	–	**21**
D. (*S.*) *auraria*	–	–	–	–	–	–	–	1	–	21	–	–	–	**22**
D. (*S.*) *rufa*	18	26	45	1	5	1	–	55	25	301	3	–	9	**489**
D. (*Drosophila*) *brachynephros*	8	–	–	–	–	–	–	–	–	11	–	–	–	**19**
D. (*D.*) *bizonata*	61	13	10	–	6	2	1	6	3	66	4	6	8	**186**
D. (*D.*) *sternopleuralis*	–	3	7	2	3	1	1	1	6	–	–	1	5	**30**
D. (*D.*) *albomicans*	1	2	2	–	–	–	–	–	–	–	–	–	1	**6**
D. (*D.*) *annulipes*	2	–	2	2	1	–	–	–	1	–	–	–	–	**8**
D. (*D.*) *curviceps*	–	–	–	–	–	–	2	27	–	–	–	–	–	**29**
D. (*D.*) *immigrans*	–	2	11	–	–	–	1	39	7	2	–	–	–	**62**
D. (*Dorsilopha*) *busckii*	–	–	–	–	–	–	–	4	–	–	–	–	–	**4**
Total number of individuals	215	226	334	51	81	22	19	244	80	565	13	46	104	**2000**
Number of species	21	18	19	8	9	7	11	18	17	18	6	6	11	**43**
Number of collections	8	3	8	4	4	3	4	4	4	4	4	4	4	

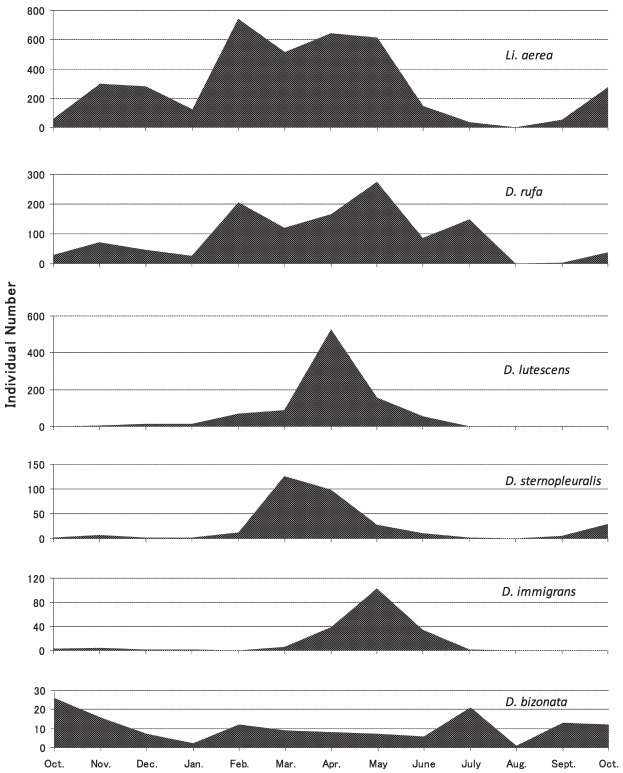

Figure 1 : Seasonal fluctuation patterns of six dominant drosophilid species collected from October, 2009 to October, 2010 at Dogo Park.

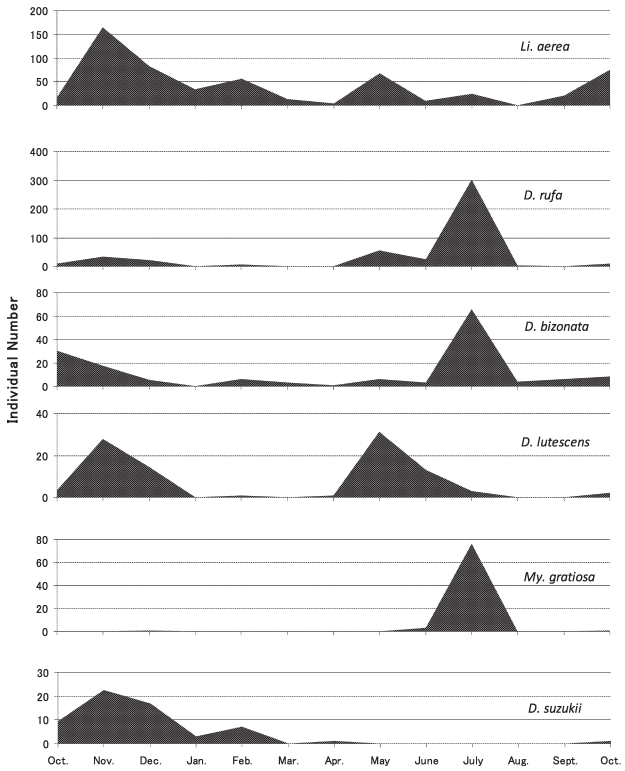

Figure 2 : Seasonal fluctuation patterns of six dominant drosophilid species collected from October, 2009 to October, 2010 on Mt. Miyukiji.

Table 3 : Number of drosophilid flies collected in Matsuyama General Park

Year	2009	2010		
Drosophilid species/Month	Oct.	Apr.	May	**Total**
Steganinae				
Leucophenga angusta	1	–	1	**2**
L. maculata	1	–	–	**1**
L. orientalis	3	–	–	**3**
Drosophilinae				
Liodrosophila aerea	13	–	1	**14**
Paramycodrosophila nakamurai	–	–	1	**1**
Scaptomyza (*Parascaptomyza*) *pallida*	–	9	–	**9**
Drosophila (*Sophophora*) *suzukii*	–	2	–	**2**
D. (*S.*) *lutescens*	–	1	12	**13**
D. (*S.*) *ficusphila*	1	–	–	**1**
D. (*S.*) *rufa*	6	–	–	**6**
Drosophila (*Drosophila*) *guangdongensis*	1	–	–	**1**
D. (*D.*) *bizonata*	13	–	1	**14**
D. (*D.*) *sternopleuralis*	–	–	2	**2**
D. (*D.*) *annulipes*	1	–	–	**1**
D. (*D.*) *curviceps*	–	1	1	**2**
D. (*D.*) *immigrans*	–	–	1	**1**
Total number of individuals	40	13	20	**73**
Number of species	9	4	8	**16**

only *A. furcata*. Therefore, the remaining female specimens would be of *A. furcata* as well. Combining these results with the previous records by Okada (1988), Watada et al. (2000) and Hoshina and Watada (2001), a list of 79 drosophilid species belonging to 16 genera so far recorded from Ehime Prefecture is presented in Table 4. Of them, two are undescribed new species. *Phortica* sp. aff. *glabra* belongs to the *P. foliiseta* species complex that is distributed in subtropical and tropical regions from Taiwan to New Guinea (Toda, 2006–2010); only one species, *P. speculum* (Máca & Lin, 1993), has been recorded from Japan (Iriomote-jima). *Scaptodrosphila* sp. is widely distributed in western Japan (Watada, unpublished data). A new record from Japan is *D. guangdongensis* collected in Matsuyama General Park. In addition, the following seven species were newly recorded from Shikoku: *Stegana nigrifrons*, *Microdrosophila pseudopleurolineata*, *Liodrosophila castanea*, *Dichaetophora delicata*, *Mycodrosophila planipalpis*, *Zaprionus aungsani* and *Hirtodrosophila mediohispida*. *Drosophila takahashii*, which had been recorded in southern Ehime (Watada et al., 2000) and Kochi (Okada, 1988), was collected from Matsuyama for the first time in the summer of 2010, suggesting that its distribution range is expanding to north in Shikoku.

Liodrosophila aerea was the most dominant species, occupying 53% of the total sample collected at Dogo Park and 31% on Mt. Miyukiji. Such extraordinary abundance of this species is a remarkable characteristic of the samples obtained by net

Table 4 : Drosophilid species recorded in Ehime Prefecture, Shikoku

	Species	References†		Species	References†
1	*Stegana nigrifrons* de Meijere, 1911*		41	*H. histrioides* (Okada & Kurokawa, 1957)	1)
2	*Amiota furcata* Okada, 1971		42	*H. mediohispida* (Okada, 1967)*	
3	*Phortica magna* (Okada, 1960)		43	*H. omogoensis* (Okada, 1956)	1)
4	*P. okadai* (Máca, 1977)		44	*H. quadrivittata* (Okada, 1956)	1)
5	*P.* sp. aff. *glabra****		45	*Drosophila* (*Sophophora*) *bifaciata* Pomini, 1940	3)
6	*Leucophenga actipollinosa* Okada, 1987	1)	46	*D.* (*S.*) *tukubaensis* Takamori & Okada, 1983	
7	*L. angusta* Okada, 1956		47	*D.* (*S.*) *oshimai* Choo & Nakamura, 1973	
8	*L. bellula* (Bergroth, 1894)		48	*D.* (*S.*) *subpulchrella* Takamori & Watabe, 2006,	
9	*L. concilia* Okada, 1956		49	*D.* (*S.*) *suzukii* (Matsumura, 1934)	
10	*L. interrupta* Duda, 1924		50	*D.* (*S.*) *lutescens* Okada, 1975	
11	*L. maculata* (Dufour, 1839)		51	*D.* (*S.*) *takahashii* Sturtevant, 1927	
12	*L. orientalis* Lin & Wheeler, 1972		52	*D.* (*S.*) *melanogaster* Meigen, 1830	
13	*L. ornata* Wheeler, 1959		53	*D.* (*S.*) *simulans* Sturtvant, 1919	
14	*Microdrosophila* (*Microdrosophila*) *maculata* Okada, 1960		54	*D.* (*S.*) *ficusphila* Kikkawa & Peng, 1938	
15	*Mi.* (*Mi.*) *pseudopleurolineata* Okada, 1968*		55	*D.* (*S.*) *auraria* Peng, 1937	
16	*Mi.* (*Oxystyloptera*) *urashimae* Okada, 1960		56	*D.* (*S.*) *biauraria* Bock & Wheeler, 1972	
17	*Liodrosophila aerea* Okada, 1956		57	*D.* (*S.*) *kikkawai* Burla, 1954	1)
18	*Li. castanea* Okada & Chung, 1960*		58	*D.* (*S.*) *rufa* Kikkawa & Peng, 1938	
19	*Dichaetophora acutissima* (Okada, 1956)		59	*D.* (*S.*) *triauraria* Bock & Wheeler, 1972	1)
20	*Di. delicata* (Nishiharu, 1981)*		60	*D.* (*Drosophila*) *guangdongensis* Toda & Peng, 1989**	
21	*Di. tenuicauda* (Okada, 1956)	1)	61	*D.* (*D.*) *virilis* Sturtevant, 1916	2)
22	*Mycodrosophila gratiosa* (de Meijere, 1911)		62	*D.* (*D.*) *tsigana* Burla & Gloor, 1952	1)
23	*My. palmata* Okada, 1956	1)	63	*D.* (*D.*) *lacertosa* Okada, 1956	2)
24	*My. planipalpis* Kang, Lee & Bahng, 1966*		64	*D.* (*D.*) *neokadai* Kaneko & Takada, 1966	3)
25	*My. poecilogastra* (Loew, 1874)		65	*D.* (*D.*) *hydei* Sturtvant, 1921	
26	*My. shikokuana* Okada, 1956	1)	66	*D.* (*D.*) *daruma* Okada, 1956	2)
27	*Styloptera nishiharui* Okada, 1982	3)	67	*D.* (*D.*) *angularis* Okada, 1956	1)
28	*Paramycodrosophila nakamurai* Okada, 1973		68	*D.* (*D.*) *brachynephros* Okada, 1956	
29	*Collessia kirishimana* (Okada, 1967)		69	*D.* (*D.*) *nigromaculata* Kikkawa & Peng, 1938	
30	*Scaptomyza* (*Parascaptomyza*) *elmoi* Takada, 1970	1)	70	*D.* (*D.*) *unispina* Okada, 1956	3)
31	*Sc.* (*Para.*) *pallida* (Zetterstedt, 1847)		71	*D.* (*D.*) *testacea* von Roser, 1840	1)
32	*Sc.* (*Scaptomyza*) *consimilis* Hackman, 1955	1)	72	*D.* (*D.*) *bizonata* Kikkawa & Peng, 1938	
33	*Sc.* (*Sc.*) *graminum* (Fallén, 1823)		73	*D.* (*D.*) *histrio* Meigen, 1830	1)
34	*Zaprionus* (*Anaprionus*) *aungsani* Soe Wynn & Toda, 1988*		74	*D.* (*D.*) *sternopleuralis* Okada & Kurokawa, 1957	
35	*Z.* (*A.*) *grandis* (Kikkawa & Peng, 1938)	1)	75	*D.* (*D.*) *albomicans* Duda, 1924	
36	*Scaptodrosophila coracina* (Kikkawa & Peng, 1938)		76	*D.* (*D.*) *annulipes* Duda, 1924	
37	*Sa. subtilis* (Kikkawa & Peng, 1938)		77	*D.* (*D.*) *curviceps* Okada & Kurokawa, 1957	
38	*Sa. throckmortoni* (Okada, 1973)	1)	78	*D.* (*D.*) *immigrans* Sturtvant, 1921	
39	*Sa.* sp.***		79	*D.* (*Dorsilopha*) *busckii* Conquillett, 1901	
40	*Hirtodrosophila alboralis* (Momma & Takada, 1954)	1)			

† References: 1) Okada (1988), 2) Watada et al. (2000), 3) Hoshina and Watada (2001); unless noted, the species was recorded in this study. New records: *new to Shikoku, **new to Japan, ***new species.

sweeping in the surveyed area, compared with samples from other areas such as the Imperial Palace of Tokyo (Beppu, 2006) and Iriomote-jima of the Ryukyu Islands (Hirai et al., 2000), where *Li. aerea* occupied only 1.6% and 1.8%, respectively. The second abundant species was *D. rufa* (17%), followed by *D. lutescens* (9.6%), *D. sternopleuralis* (4.0%) and *D. bizonata* (3.8%). The relative frequencies of these species are similar to those observed in the Imperial Palace (Beppu, 2006), where *D. rufa* occupied 14%, *D. lutescens* 8.5%, *D. sternopleuralis* 6.8% and *D. bizonata* 4.3%.

In spite of the five times difference in the total number of collected individuals between Dogo Park (9,590) and Mt. Miyukiji (2,000), the total numbers of species were nearly the same, 45 species in Dogo Park and 43 species on Mt. Miyukiji. However, the species composition was somewhat different between the two localities, with ten species (*Leucophenga concilia, Microdrosophila maculata*, *Mi. urashimae*, *Li. castanea*, *Z. aungsani*, *D. tsukubaensis*, *D. simulans*, *D. biauraria*, *D. hydei* and *D. nigromaculata*) collected only in Dogo Park and six species (*St. nigrifrons*, *A. furcata*, *P.* sp. aff. *glabra*, *Di. delicata*, *My. planipalpis* and *My. poecilogastra*) collected only on Mt. Miyukiji. A part of these faunal characteristics reflected differences in richness of the subfamily Steganinae and the genus *Mycodrosophila* between the two localities: 73 individuals representing nine species of two steganine genera and 12 individuals of one species of *Mycodrosophila* were collected in Dogo Park, whereas 121 individuals of 11 species of four steganine genera and 107 individuals of three species of *Mycodrosophila* on Mt. Miyukiji. The richness of these species on Mt. Miyukiji

should be due to abundance of their food substances such as leaves, tree sap and fungi there.

3.2 Seasonal changes

Seasonal changes in the number of individuals collected per month at Dogo Park and on Mt. Miyukiji are shown, separately for each of the six dominant species at each locality, in Figs 1 and 2, respectively. *Liodrosophila aerea*, the most dominant species, was abundant in the spring and the fall, though two times more in the spring than in the fall, at Dogo Park, but was abundunt only in the fall on Mt. Miyukiji. *Drosophila rufa* was similar to *Li. aerea* in the seasonal fluctuation pattern at Dogo Park, but was quite different from it on Mt. Miyukiji showing only one peak in July. The patterns of *D. lutescens* and *D. bizonata* were also different between the two localities. *Drosophila lutescens* was abundant in November and May on Mt. Miyukiji, but had a large peak in April at Dogo Park. On Mt. Miyukiji, the numbers of collected individuals of *D. bizonata* and *My. gratiosa* were the most abundant in July of 2010. Most individuals of these species were collected from white fungi that grew on fallen trees. *Drosophila sternopleuralis* and *D. immigrans* showed a peak in March and in May, respectively, at Dogo Park. *Drosophila suzukii* occured from the fall to the winter, being relatively abundant in the fall, on Mt. Miyukiji.

The number of collected flies in August and September of 2010 was the least among the 13 months of survey period. According to the records of Matsuyama Local Meteorological Observatory, the monthly mean air temperature of August, 2010 in Matsuyama was 29.7 ℃ and the monthly precipitation was 4.5 mm, being the highest and the third least, respectively, since the first observation in 1890 there. The drastic decrease of collected flies in August and September of 2010 seems to have reflected this extraordinarily severe condition. Even in usual summers, the population densities of many drosophilid species decrease at lowlands of central mainland of Japan, and some species such as *D. oshimai* and *D. suzukii* are considered to migrate from lowlands to highlands in summer (Beppu, 2006). Likely, some species would have escaped from the severe summer conditions by migrating from the collection localities to the backyard mountain range.

Usually, banana-bait trapping is not so effective to collect drosophilid flies in mid winter even in relatively warm areas of central to southern Japan (e.g., Toda, 1979; Watada et al., 2000): Toda (1979) collected drosophilid flies in January at Kariya, Aichi Prefecture by banana-bait trapping and net sweeping, and obtained 219 individuals by net sweeping and 20 individuals by trapping. This suggests that net sweeping is more effective than banana-bait trapping in winter in the mainland of Japan. In the present study as well, drosophilid flies collected from January to February at Dogo Park were more than those in August and September of 2010 there; many *Camellia japonica* trees bloomed from the late January to March at Dogo Park, and a lot of drosophilid flies were collected from March to May on its fallen flowers. Most individuals of *D. lutescens* and *D. sternopleuralis* were collected from the fallen flowers in winter at Dogo Park. On the other hand, only few drosophilid flies were collected in March and April was on Mt. Miyukiji, probably due to lack of *C. japonica* trees there.

We collected many drosophilid flies at the two localities of Matsuyama by year-round net sweeping, with discovery of the following new records: two species new to science, one to Japan and seven to Shikoku. In the present study, however, seasonal population fluctuation patterns were preliminarily described only for eight dominant species, based on samples collected by net sweeping conducted in the not strictly quantitative way. It is necessary to conduct sampling in more quantitive ways, for example by standardizing the number of sweeps per sampling, and to analyze the reproductive age structure of populations in order to understand the phenological properties of drosophilids in a warm-temperate region of southern Japan. In addition, more extensive surveys in different environments will bring more new findings of drosophilid faunal components in Ehime Prefecture.

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キノコ食ショウジョウバエ類とその寄生性センチュウとの関係

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キノコ食ショウジョウバエ類に対するセンチュウの寄生が北米，ヨーロッパなどで調査され，その効果はショウジョウバエ種間及び地域間で大きく異なることが報告されている．今回，北海道大学苫小牧研究林において 4 年間，キノコ上のショウジョウバエ成虫を採集し，センチュウによる寄生の有無と，寄生が蔵卵数に及ぼす影響を調べた．その結果，寄生率はショウジョウバエ種間，季節間，年次間で大きく異なること，寄生はどのショウジョウバエ種においても不妊化をもたらすことが明らかになった．また，ショウジョウバエ種の相対頻度と寄生率間には相関は認められなかった．このことは，ショウジョウバエ類の共存へのセンチュウ寄生の寄与は大きくないことを示している．

Relationship between mycophagous drosophilid flies and their parasitic nematodes

Hiroshi Yorozuya[1]

The prevalence of nematode parasitism in mycophagous drosophilids was studied throughout their active season over 4 years (2000–2003) in Tomakomai Experimental Forest of Hokkaido University, northern Japan. In addition, effects of nematode parasitism on the feritility of drosophilid females were examined. A total of 18,177 adult drosophilid flies were collected from 43 fungal species found on the census route. The rate of nematode parasitism varied yearly, seasonally and among drosophilid species. Parasitized females carried much fewer eggs than unparasitized ones, revealing a strong deleterious effect of parasitism on the fitness of flies. The relative abundance of drosophilid species and the rate of parasitism were not significantly correlated, suggesting that nematode parasitism unlikely promoted the coexistence of drosophilid flies in this community.

1．はじめに

寄生者は，食物網の高次栄養段階に属し，宿主の群集構造に直接的・間接的に強い影響を与えるなど，生態系内で非常に重要な役割を持っている（Freeland, 1983; Minchella and Scott, 1991; Dobson and Clawley, 1994; Marcogliese and Cone, 1997; Jaenike and Perlman, 2002）．センチュウは，真菌や寄生蜂と同じく昆虫類の主要な寄生者であるが，自然条件下で宿主の個体群および群集構造に与える効果を検証した事例は多くない．多くのキノコ食ショウジョウバエ種もセンチュウの寄生を受け，産卵数の減少，成虫の死亡，オスの交尾行動の阻害などを通し，適応度が低下することが知られている（Welch, 1959; Montague and Jaenike, 1985; Jaenike, 1988; Jaenike et al., 1995; Jaenike and Perlman, 2002）．また，センチュウ寄生が個体群密度や群集構造に与える影響については北アメリカ（Jaenike, 1992; Jaenike and Perlman, 2002）及びヨーロッパの各地域（Klarenberg and Kersten, 1994; Gillis and Hardy, 1997）で調査されており，寄生率がショウジョウバエ種間及び地域間で大きく異なることが明らかになっている．このことは，センチュウ寄生がショウジョウバエ群集構造に影響する効果の大きさを地域間で比較する必要があることを示唆している．

また，ヨーロッパにおいてセンチュウの寄生率は，各キノコ食ショウジョウバエ種の相対頻度に相関することが報告されている（Gillis and Hardy, 1997; Hardy and Gillis, 1998）．このことは，センチュウが，優占するショウジョウバエ種の個体群密度を抑制し，ショウジョウバエ種間の競争を緩和することで，ショウジョウバエ類の共存を促進している可能性があることを示唆しており，この共存のメカニズムは〝disproportionate parasitism hypothesis（DPH）〟と名づけられている（Gillis and Hardy, 1997; Hardy and Gillis, 1998）．本論文で

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は，Yorozuya（2009）が報告した北海道大学苫小牧研究林における4年間の調査結果を，北アメリカやヨーロッパ各地域での結果と比較して，1）寄生率のショウジョウバエ種間，季節・年次間変動の傾向を明らかにしその要因について考察すること，2）地域間およびショウジョウバエ種間での不妊化効果を比較すること，3）日本を含む各地域におけるDPHを評価することを目的とした．

2．キノコ食ショウジョウバエ類に寄生するセンチュウの生活史

キノコ食ショウジョウバエ類に寄生する主なセンチュウは，*Howardula aoronymphium* Welch, 1959および*Howardula* sp.である（Jaenike and Perlman, 2002; Perlman and Jaenike, 2003）．Welch（1959）が記述しているように，センチュウの成虫はアルファベットのCの形をしており，乳白色をしている．メス成虫の大きさは，幅が約0.16 mm，長さが約1.5 mm程度で肉眼でも確認できるほどの大きさであるが，オス成虫は，幅が約0.2 mmで長さが約0.5 mm程度である．幼虫は，ほぼ透明で体長が0.2 mmから0.4 mm程度である．*Howardula*属のセンチュウを形態により同定することは非常に困難であるので，Jaenike and Perlman（2002）とPerlman and Jaenike（2003）は，DNA情報によって種を同定している．日本からは，*Hirtodrosophila quadrivittata*種群や*Drosophila histrio* Meigan, 1830に寄生する*Howardula* sp.と*D. orientacea* Grimaldi et al., 1992に寄生する*H.* cf. *aoronymphium*の2種が報告されており（Jaenike and Perlman, 2002; Perlman and Jaenike, 2003），Yorozuya（2009）が報告したセンチュウもこの両種，あるいはそのどちらかであると思われる（正確な種同定は行われなかった）．

交尾したメスのセンチュウは，吻針でクチクラを突き破ってショウジョウバエ幼虫の体内に侵入し，血体腔内で成長し親虫になる．ショウジョウバエが成虫になると，親虫となったセンチュウは宿主の血体腔内におおよそ500個体の子虫を放出する．これらの子虫は，宿主のショウジョウバエ成虫が採餌や交尾のためにキノコを訪れた時に，産卵管や肛門からキノコ上に脱出し，交尾をし，新しい宿主を探す．このように，これらのセンチュウは他に中間宿主を持たず，宿主であるキノコ食ショウジョウバエと寄生場所としてのキノコに完全に依存した生活史を送る（Welch, 1959; Jaenike and Perlman, 2002）．

3．寄生率の季節間変動と年次間変動

Yorozuya（2009）は，日本の北部冷温帯林での調査結果を報告した．調査は，北海道大学苫小牧研究林内（42°40′N，141°36′E）の9ヘクタールの成熟林分において，約2 kmの調査トランセクトを設置し，2000年から2003年まで行われた．毎回のセンサスでは，トランセクトの両脇5 m以内に発生したすべてのキノコパッチについて，その種類が記録され，パッチ上のショウジョウバエ成虫が捕虫網を用いて採集された．4年間のセンサスの結果，43種類のキノコ（Yorozuya, 2006）から18177個体のショウジョウバエ成虫が採集された（表1）．そのうちの一部（表2）がセンチュウ寄生の有無を調べるために実体顕微鏡下で解剖された．その結果，センチュウによる寄生率は，2000年が6.2%，2001

表1：北海道大学苫小牧研究林内に設定された調査トランセクト上のすべてのキノコパッチから採集されたショウジョウバエ種の成虫数と相対頻度．2000〜2002年は，キノコの子実体の発生が始まる6月から子実体の発生が終わる10月末まで，2003年は9月末まで，悪天候の日を除いてほぼ毎日行われたセンサスの結果による．採集されたショウジョウバエ成虫は，カーレ液で固定後80%エタノールで保存され，BioCIS（Biological Classification and Identification System; http://biokey.museum.hokudai.ac.jp/Classification/index.jsp）上の「北海道産ショウジョウバエ類」検索データベースとBeppu et al.（1977）を使って種まで同定された．〔Yorozuya（2009）から許可を得て一部改変の上転載〕

調査年：	2000		2001		2002		2003		合　計	
ショウジョウバエ種	採集数	相対頻度	採集数	相対頻度	採集数	相対頻度	採集数	相対頻度	採集数	相対頻度
Hirtodrosophila sexvittata (Okada)	2195	0.459	3183	0.669	3705	0.543	896	0.495	9979	0.549
Hirtodrosophila trilineata (Chung)	953	0.199	988	0.208	2647	0.388	745	0.412	5333	0.293
Hirtodrosophila histrioides (Okada & Kurokawa)	137	0.029	155	0.033	269	0.039	82	0.045	643	0.035
Hirtodrosophila alboralis (Momma & Takada)	934	0.195	103	0.022	47	0.007	10	0.006	1094	0.060
Drosophila unispina Okada	162	0.034	87	0.018	23	0.003	15	0.008	287	0.016
Drosophila orientacea Grimaldi *et al*.	166	0.035	104	0.022	43	0.006	43	0.024	356	0.020
Mycodrosophila takachihonis Okada	45	0.009	40	0.008	45	0.007	0	0.000	130	0.007
Mycodrosophila atrithorax Okada	31	0.006	74	0.016	8	0.001	3	0.002	116	0.006
Drosophila histrio Meigan	11	0.002	6	0.001	29	0.004	14	0.008	60	0.003
その他*	146	0.031	19	0.004	13	0.002	1	0.001	179	0.010
合計	4780		4759		6829		1809		18177	

* 4年間の総採集個体数が50個体以下の非優占種．

表 2：北海道大学苫小牧研究林で採集されたキノコ食ショウジョウバエ種のセンチュウによる寄生率．表 1 の成虫の一部を解剖して調べた．〔Yorozuya（2009）から許可を得て一部改変の上転載〕

調査年：	2000		2001		2002		2003		合　計	
ショウジョウバエ種	解剖数	寄生率	解剖数	寄生率	解剖数	寄生率	解剖数	寄生率	解剖数	寄生率
H. sexvittata	867	0.054	957	0.049	1330	0.034	621	0.023	3775	0.041
H. trilineata	451	0.047	884	0.053	873	0.021	477	0.025	2685	0.036
H. histrioides	89	0.112	142	0.085	240	0.017	65	0.092	536	0.060
H. alboralis	282	0	96	0.010	35	0	10	0	423	0.002
D. unispina	136	0.162	65	0.108	16	0.125	15	0.067	232	0.138
D. orientacea	164	0.177	79	0.089	39	0.103	43	0.070	325	0.132
M. takachihonis	25	0	39	0	21	0	0	0	85	0
M. atrithorax	24	0	74	0	7	0	3	0	108	0
D. histrio	11	0.091	6	0	25	0.160	14	0.143	56	0.125
その他*	50	0	16	0	12	0	0	0	78	0
合計	2103	0.062	2358	0.051	2598	0.030	1248	0.030	8307	0.044

* 総解剖数が 50 個体以下の非優占種．

表 3：センチュウ寄生率の変動に寄与する要因．表 2 のデータを用いた一般化線形モデルによる検定：応答変数は，各ショウジョウバエ個体のセンチュウ寄生の有無（1 もしくは 0）；説明変数は，種，採集された月および調査年次；各ショウジョウバエ個体の寄生の有無の確率分布は，二項分布を仮定；R　2.4.1 software, R Development Core Team, Viena, Austria を用いて解析．〔Yorozuya（2009）から許可を得て翻訳の上転載〕

説明変数	d.f.	Sum of squares	Mean square	*F*	*P*
年次	1	2.14	2.14	53.13	<0.0001
月	4	2.22	0.55	13.80	<0.0001
ショウジョウバエ種	9	5.77	0.64	15.95	<0.0001
年次×月	4	0.44	0.11	2.77	0.026
年次×ショウジョウバエ種	9	0.75	0.08	2.08	0.028
月×ショウジョウバエ種	35	1.82	0.05	1.29	0.115
年次×月×ショウジョウバエ種	29	1.20	0.04	1.03	0.417
残差	8211				

年が 5.1%，2002 年と 2003 年が 3.0%であり（表 2），年次間の差は統計的に有意であった（表 3）．また，寄生率は，各年とも 6 月から 7 月に高く，9 月から 10 月に低くなる傾向を示し（図 1），月間における差も統計的に有意であった（表 3）．このように，北海道におけるセンチュウの寄生率は，年次変動とともに，春に高く季節がすすむにつれて減少する季節変動を示す．こうしたキノコ食ショウジョウバエ類におけるセンチュウ寄生率の季節変動は，北アメリカでは春は平均の寄生率が 16%程度と高いが，夏になると 10%程度にまで低くなり，夏の終わりから秋にかけて 20%程度にまで高くなる傾向があることが報告されている（Jaenike, 1992; Jaenike and Perlman, 2002）．しかしオランダでは，顕著な寄生率の季節変動はないものの，春から季節がすすむにつれてやや低くなる傾向があると報告されている（Gillis and Hardy, 1997）．寄生率の年次変動も大きく，年によって 8.4%から 19.3%にまで変動することが，北アメリカの個体群で明らかにされている（Jaenike, 2002）．Jaenike（2002）は，降雨量や平均気温などの気象要因の変動がキノコ子実体の発生量やショウジョウバエの個体群サイズの変動をもたらしていると指摘している．また，Yorozuya（2006）も，苫小牧研究林において季節および年によりキノコの発生量やショウジョウバエの個体群密度が変動することを報告している．こうしたキノコの発生量やショウジョウバエの個体群密度の変動が寄生率にどのように影響するかまだはっきりしないが，キノコパッチを訪れるショウジョウバエ数が増えると，センチュウによって寄生されているショウジョウバエの訪問数が増加し，そのキノコパッチ内で発育する

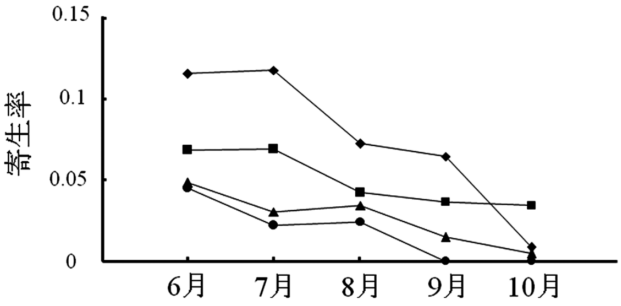

図 1：北海道大学苫小牧研究林におけるキノコ食ショウジョウバエ（全種）に対するセンチュウによる寄生率の季節変動．各年のシンボルはそれぞれ◆，2000；■，2001；▲，2002；●，2003 を示す．〔Yorozuya（2009）から許可を得て翻訳の上転載〕

ショウジョウバエ幼虫が寄生される確率が高まる可能性がある（Jaenike, 1992; Jaenike and Perlman, 2002）．また，キノコパッチの数や大きさ，パッチ内の幼虫密度などの要因も寄生率の変動に関与していると思われる．今後，各季節の各種のキノコパッチから発生したショウジョウバエの個体数とその寄生率，キノコを訪ずれた成虫数とその寄生率，キノコパッチや資源量の季節・年次変動に関するデータも加え，どういった要因が寄生率の変動に関わっているかを解析することが望まれる．

4．各ショウジョウバエ種における寄生率

Yorozuya（2009）の解析結果は，ショウジョウバエ種間の寄生率の差も統計的に有意であった（表3）．寄生率は *D. unispina* Okada, 1956 と *D. orientacea* および *D. histrio* で高く，優占種である *H. sexvittata*（Okada, 1956）と *H. trilineata*（Chung, 1960），非優占種の *H. histrioides*（Okada & Kurokawa, 1957）では中程度で，一方 *Mycodrosophila atrithorax* Okada, 1986 と *M. takachihonis* Okada, 1956 は，全く寄生されていなかった（表2）．このように，他地域における研究と同じく，日本においてもショウジョウバエ種間でセンチュウによる寄生率に大きな差があることが明らかになった．Montague and Jaenike（1985）は，*D. testacea* 種群の *D. neotestacea* Grimaldi et al., 1992 と *D. putrida* Sturtevant, 1916 は，*D. quinaria* 種群の *D. falleni* Wheeler, 1960 や *D. recens* Wheeler, 1960 よりもセンチュウによる寄生率が高いことを報告している．彼らは，*testacea* 種群のショウジョウバエ種は，*quinaria* 種群のものよりも，センチュウの密度がより高いと考えられる古くて腐ったキノコに産卵する傾向があり，そのために被寄生率が高くなるのではないかと考えている．しかし，Klarenberg and Kersten（1994）と Gillis and Hardy（1997）は，*D. testacea* van Roser, 1840 の被寄生率は，*quinaria* 種群のショウジョウバエ種よりも低いと報告している．また，Kimura and Toda（1989）は，センチュウの寄生率は，新鮮なキノコを利用するショウジョウバエ種と腐ったキノコを利用するショウジョウバエ種の間で明確な差がないことを報告している．これらのことから，ショウジョウバエのキノコの腐敗ステージに対する選好性がセンチュウによる寄生率に影響するかどうかは，まだ結論できず，さらなる研究が必要である．また，キノコパッチの大きさは，寄生率に影響する要因になりうるのではないかと思われる．ウスヒラタケ *Pleurotus pulmonarius* Quel. やヌメリツバタケ *Oudemansiella mucida* Hounel は，多数の子実体が集まった大きなパッチで発生し，そこから多数の *H. sexvittata* や *H. trilineata* が発生する（Yorozuya, 2006）．大きなキノコパッチ内の子実体間にショウジョウバエ幼虫とセンチュウが独立に集中分布すれば，センチュウがほとんどいない子実体が寄生からの空間的な逃避場所となり，こうした逃避場所を含む大きなキノコパッチから主に発生する *H. sexvittata* や *H. trilineata* の寄生率が低くなる可能性がある．また，それぞれの地域で，*M. atrithorax* や *M. takachihonis* のようにセンチュウに全く寄生されないショウジョウバエ種がいることがわかっている．*Hirtodrosophila alboralis*（Momma & Takada, 1954）は，*H. sexvittata* や *H. trilineata* と同様にヒラタケ属をはじめとする多種類のキノコ種から発生するが（Kimura and Toda, 1989），Yorozuya（2009）の調査では，寄生率がわずか0.2%にすぎなかった．これらのショウジョウバエ種は，センチュウの寄生に対して抵抗性があるかもしれない．Welch（1959）と Jaenike（1992）は *Howardula aoronymphium* の接種実験を行い，*D. buskii* Coquillett, 1901 や *D. quinaria* Loew, 1866 は寄生されるが，*D. immigrans* Sturtevant, 1921 や *D. obscura* Fallén, 1823 は寄生されないことを報告している．

5．DPHの検証

Yorozuya（2009）は，Gillis and Hardy（1997）の方法に従ってDPH（disproportionate parasitism hypothesis）について検証し，すべての調査年で各ショウジョウバエ種の相対頻度と寄生率には正の相関がないことを示した（2000年：$\tau=0.156$，$P=0.53$；2001年：$\tau=0.378$，$P=0.13$；2002年：$\tau=0.111$，$P=0.66$；2003年：$\tau=0.333$，$P=0.18$）．また，Fisher's combined probability method を用い，4年間合計したデータについても，相対頻度と寄生率には正の相関がないことを示した（$\chi_8^2=9.65$，$P=0.31$）．つまり，北日本のキノコ食群集では，DPHは支持されなかった．同様にドイツ，スコットランドおよびアメリカのショウジョウバエ群集においてもDPHは支持されなかった（Gillis & Hardy 1997; Hardy & Gillis 1998）が，オランダ，デンマークおよびイングランドでは，DPHを支持する結果が得られている（Gillis & Hardy 1997; Hardy & Gillis 1998）．このように，センチュウによるトップダウン効果，すなわちショウジョウバエ種間の競争を緩和することで共存を促進する効果は，地域により異なるようである．日本の群集でDPHが支持されなかった理由として，前述したように優占する *H. sexvittata* と *Hi. trilineata* がどちらも大きなパッチを形成するヌメリツバタケやウスヒラタケを主要な繁殖資源として利用するために寄生率が高くならないことが考えられる．各地域のショウジョウバエが利用するキノコ種の特性によって，センチュウ寄生によるトップダウン効果がショウジョウバエ群集構成種の共存を促進するかどう

かが決まる可能性がある．

6．センチュウによるメスの不妊化の効果

Yorozuya（2009）は，Jaenike（1992）と同様に，卵巣内の成熟した卵（ステージ11かそれ以降の成熟度；Mahowald and Kambysellis, 1980）を数え，ショウジョウバエ種ごとにセンチュウに寄生されたメスとされていないメスで蔵卵数に差があるかどうか調べた．その結果，センチュウに寄生されたメスの蔵卵数は0から4個程度であり，寄生されていないメスと比べて統計的に有意に低かった（図2）．この結果は，センチュウによる寄生がほぼ完全な不妊化を引き起こすことを示している．他の地域における研究では，センチュウによる寄生は必ずしもショウジョウバエに不妊化をもたらすものではないことが報告されている．例えば，*testacea* 種群の *D. neotestacea* と *D. putrida* はセンチュウ寄生によりほぼ完全に不妊化するものの，*quinaria* 種群の *D. falleni* と *D. recens* では，寄生されたメスはされていないメスの半数程度の卵を持ち，不妊化の効果がより小さい（Jaenike, 1992; Jaenike and Perlman, 2002）．さらに，オランダやドイツの *D. testacea* の個体群は，寄生により不妊化することはほとんどない（Gillis & Hardy 1997）．しかし，日本では，センチュウ寄生はどのショウジョウバエ種に対してもほぼ完全な不妊化をひき起こして，適応度に大きな影響を与えていた．

7．まとめ

本論文では，北海道における調査結果を紹介したうえで，北アメリカやヨーロッパ各地域での結果と比較して，ショウジョウバエ種間および季節・年次間の寄生率の違い，地域間でのショウジョウバエ類の共存促進効果の違いおよび地域・ショウジョウバエ種間における不妊化効果に違いがあることを示した．不妊化効果の違いを生む要因については，十分に論じられなかったが，その他の違いについては，キノコパッチの大きさやキノコ発生量の変動など各種キノコパッチごとの特性が寄与する可能性を指摘した．今後，キノコ資源量の季節・年次変動に関するデータや各季節の各種のキノコパッチから得られた情報，例えば，キノコパッチの大きさ，発生したショウジョウバエの個体数とその寄生率などの情報を加えて，どういった要因が寄生率の変動に関わっているかを解析する必要がある．

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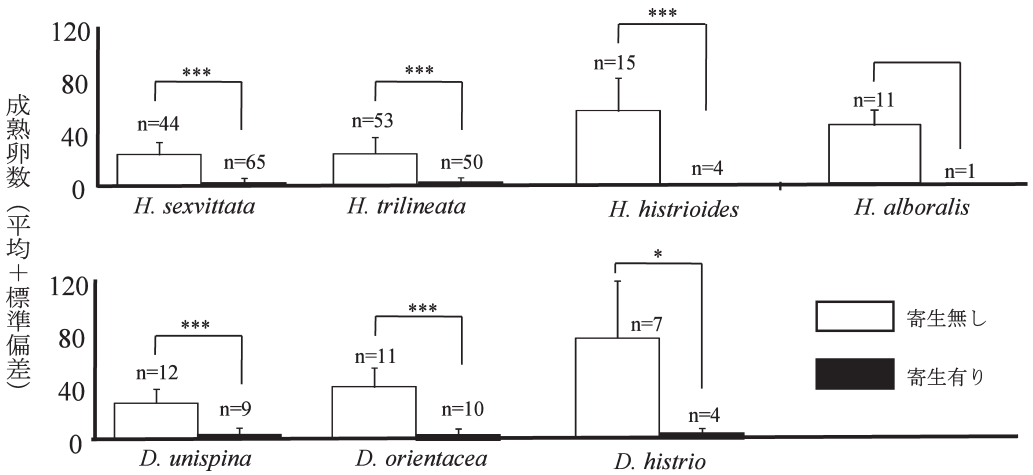

図2：ショウジョウバエ種ごとのセンチュウに寄生されたメスと寄生されていないメスの成熟卵数（平均＋標準偏差）の違い．成熟卵数の計測は，各ショウジョウバエ種のランダムに選んだ個体について行った．成熟卵数の差の検定は，マン・ホイットニーのU検定を用いて行った．有意水準のシンボルはそれぞれ，$*P<0.05$．$**P<0.01$，$***P<0.001$ を示す．〔Yorozuya（2009）から許可を得て改変の上転載〕

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昆虫群集組成解析への変性剤濃度勾配ゲル電気泳動（DGGE）法適用の試み

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微生物生態学で，環境中のメタゲノムから直接バクテリア群集を調べるために使われている変性剤濃度勾配ゲル電気泳動（DGGE）法を，近年その必要性が強く認識され，いろいろな取り組みが行われている生物多様性観測において，大量のサンプルを処理（種同定）しなければならないことが多い昆虫群集に適用できるかどうか，ショウジョウバエ類を材料としてテストした．その結果，PCR 増幅段階でのバイアスが避けられず，PCR-DGGE 法は，限られた条件のもとでの適用（例えば，構成種が既知のある範囲に限られた群集の種組成の解析・比較など）は可能ではあるものの，網羅的な生物多様性観測のための新しい手法としては問題があることがわかった．

A test for applying the method of denaturing gradient gel electrophoresis (DGGE) to analysis of insect community composition

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Applicability of the denaturing gradient gel electrophoresis (DGGE), which has been used to analyze microbial communities based on metagenomes in environments, to insect communities, as a useful technology for non-experts to easily identify component species in a large amount of samples to be obtained in various projects of biodiversity inventory and monitoring, was examined using some drosophilid species as test materials. As a result, it has been revealed that there are some problems in the process of PCR amplification for applying the PCR-DGGE method to the comprehensive observation of biodiversity, although it can be used under limited situations or conditions.

1. はじめに

近年，地球環境の変動と生物種の大量絶滅が進行する中で，生物多様性のインヴェントリーとモニタリングの必要性が強く認識され，いろいろな取り組みが行われている．例えば，国際的な生物多様性観測のネットワークとして，GEO BON: GEO Biodiversity Observation Network (http://www.earthobservations.org/geobon.shtml) があり，それを支える地域・国内ネットワークとしての AP-BON: Asia-Pacific Biodiversity Observation Network (http://sites.google.com/site/asiapacificbon/) や J-BON: Japanese Biodiversity Observation Network (http://www.j-bon.org/first_workshop/jbonws) が動き出そうとしている．

これらの生物多様性観測において，最も重要でありながら困難な問題は，種レベルの情報を如何に効率よく蓄積するかにある．生物学（生物多様性）の世紀と言われる 21 世紀のスタートを記念して設定された国際生物多様性観測年（IBOY: International Biodiversity Observation Year）に行われた，西太平洋・アジア地域生物多様性一斉観測（DIWPA-IBOY，2001～2003 年）では，観測手法を標準化して（Nakashizuka and Stork, 2002; http://diwpa.ecology.kyoto-u.ac.jp/diwpa_iboy/iboy_manuals.html）比較可能なデータを蓄積することを目指した．森林生態系の無脊椎動物の多様性観測には，東シベリアからタスマニアに至るほぼ地球を縦断するトランセクトに沿って，26 の観測サイトが設置され，標準化された方法でサンプリングを行った（戸田，2002）．しかしながら，得られた貴重かつ膨大なサンプルは，多くのサイトで，高次分類群（多くは目）レベルでソートされたにとどまり，所期の目的である種レベル

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の比較可能なデータを蓄積することが達成されたとは言い難い．

最大の障壁は，膨大なサンプルの種レベルの同定作業である．生物（昆虫）相の解明が進んでいない熱帯域に関しては，分類学的研究の進展を待つしかないが，相当程度にわかっている温帯域といえども，サンプルの量が膨大になると，種レベルの同定はそう簡単ではない．例えば，DIWPA-IBOY のコアーサイトの一つである北海道大学苫小牧研究林で，ライトトラップで採集された全（126）サンプルには，鱗翅目昆虫が 892 種，51,742 個体，鞘翅目が 355 種，11,633 個体含まれていた（Hirao et al., 2006）．これらのサンプルは，観測チームに加わっていたそれぞれの目の分類の専門家が，多大な労力と時間をかけて同定してくれたのであるが，一般に，このような作業を専門の分類学者に依頼することは困難である．この難問を解決するためには，複数の種を含んだ大量のサンプルを，迅速かつ簡便に，専門の分類学的知識に頼らないで処理するための新たな手法の開発および導入が不可欠である．

そのための手法の一つとして，形質データベースに基づいてコンピュータ上に構築される（多くの画像情報，あるいは他のデータベースへのリンクを同定の支援ツールとして活用できる），多重入力（あるいは会話）型検索システムがある：Lucid (http://www.lucidcentral.com/), DELTA: DEscription Language for TAxonomy (http://delta-intkey.com/), BioCIS: Biological Classification and Identification System (Toda et al., 2004; 戸田，2007; http://biokey.museum.hokudai.ac.jp/Classification/index.jsp)．これは，従来の二分岐検索表と異なり，検索プロセスの自由度を提供するユーザーフレンドリーな検索ツールである．また，以下に紹介する手法と異なり，ユーザーが同定作業を繰り返すことにより，生物群（種）の特徴を自然に理解し，同定者の能力開発ツールともなり得る利点を備えている．

これを一歩進めると，コンピュータ自動検索同定システムとなる．基本設計は，画像などの情報を取り込む感覚部分，取り込んだ情報を前処理する部分，形質を抽出する部分，抽出した形質によって分類する部分，分類結果を出力する部分から構成される．まさに，人間の分類作業を模したコンピュータロボットである．その中心である，形質抽出，分類過程には，ニューラルネットワークモデルが使われる．このロボットの優れている点として，感覚部分に人間にはないセンサー（例えば，コウモリのエコーロケーションに使われる高周波音声をキャッチするためのセンサー）を使うことができること，疲れないこと・飽きないこと・慣れないこと（人間の場合，これがミスにつながる），同定結果の信頼度を出力できることなどが挙げられる．既にいくつかのシステムが開発され，限られた範囲ではあるが，人間（分類学者以外）以上の成功率を挙げている（MacLeod, 2007）．

最後は，DNA 塩基配列情報の利用である．これにより，分類の専門知識を持たない研究者でも，塩基配列データベースを用いることで比較的容易に種の同定を行うことが可能となってきている．地球上の全生物種を DNA 分子マーカーによって識別しようという国際的プロジェクト〝The Barcode of Life〟（Ratnasingham and Hebert, 2007; http://www.barcodinglife.com/views/login.php）が発足しており，動物についてはミトコンドリア *COI* 遺伝子の部分配列（648 塩基対）が標準マーカーに指定され，鱗翅目，魚類，鳥類などについてデータの蓄積が始まっている．しかし，１個体ずつ DNA を抽出し，標的遺伝子マーカーを増幅し，その塩基配列を決定するのであれば，手間は通常の同定作業の比ではない．多くの種を含んだ大量のサンプルを処理するためには，もっと簡便な方法が求められる．そのような方法は，主に微生物生態学分野において発展してきた．環境中から得られる細菌のメタゲノム DNA から，細菌の種同定に使われる標準遺伝子（*16S rRNA* 遺伝子）を PCR 増幅しさまざまな解析に用いることで，同定が困難な細菌の群集組成や多様性解析が可能になってきた．一般に，環境中における微生物群集の多様性は非常に大きいことが知られている．そのため，その膨大な遺伝子情報を単純化するための方法として，変性剤濃度勾配ゲル電気泳動（DGGE: denaturing gradient gel electrophoresis）法のような手法が開発されてきた（Muyzer et al., 1993）．DGGE 法は，DNA 変性剤濃度勾配ゲルを用いて電気泳動を行うことにより，同じ長さの 2 本鎖 DNA 断片であっても塩基配列の違いによって異なる位置にバンドを分離することを可能とする手法である．このように，DGGE 法は比較的迅速かつ簡便な方法であり，個々の分類群をバンドとして可視化できるため群集構造の比較を容易に行うことができる（石井ほか，2000）．

本研究では，PCR-DGGE 法が昆虫に対しても応用できるかどうかを，DNA 情報の蓄積の多いショウジョウバエ類をモデル生物群として評価した．マーカー遺伝子としては，将来網羅的な塩基配列情報の蓄積が期待される BOLD (Barcode of Life Data Systems) の標準配列である *COI* 遺伝子を用いた．この手法の適用に成功すれば，多くの種を含んだ大量のサンプルを，１個体ずつ種同定をする大変な作業の必要がなくなり，一連の手法を習得した技術者がサンプルから種レベルのデータを短時間に得ることができるようになるものと期待される．

2．材料と方法

2.1 研究材料

本研究では，ショウジョウバエ亜科 Drosophilinae の

表 1：実験に用いたショウジョウバエ種

No.*	Genus	Subgenus	Species group	Species subgroup	Species
1	*Scaptodrosophila*		*coracina*		*coracina*
2	*Chymomyza*		*procnemis*		*procnemis*
3	*Drosophila*	*Drosophila*	*virilis*		*virilis*
4	*Drosophila*	*Drosophila*	*immigrans*	*immigrans*	*immigrans*
5	*Drosophila*	*Drosophila*	*funebris*		*funebris*
6	*Drosophila*	*Drosophila*	*repleta*	*hydei*	*hydei*
7	*Drosophila*	*Sophophora*	*melanogaster*	*melanogaster*	*yakuba*
8	*Drosophila*	*Sophophora*	*melanogaster*	*melanogaster*	*simulans*
9	*Drosophila*	*Sophophora*	*melanogaster*	*melanogaster*	*melanogaster*
10	*Lordiphosa*		*miki*		*stackelbergi*

*図 3，4 の泳動レーン番号に対応．

主な系統群を代表し，さまざまに類縁の程度が異なる 10 種を試験材料として選んだ（表 1）．それらは，最も重要なモデル生物である *Drosophila melanogaster* Meigen, 1830（キイロショウジョウバエ）とその近縁種である *Drosophila simulans* Sturtevant, 1919 および *Drosophila yakuba* Burla, 1954，比較的祖先的な *Scaptodrosophila* Duda（マメショウジョウバエ属）と *Chymomyza* Czerny（ハシリショウジョウバエ属），また，より派生的な subgenus *Drosophila* Fallén（ショウジョウバエ亜属）のいくつかの系統群などを含んでいる．

2.2　PCR プライマーの設計

ショウジョウバエ科の *COI* 遺伝子に特異的な PCR 増幅を行うために使用するプライマーを設計した．COI-a（フォワードプライマー：5’-ATCGCCTAAACTTCAGCCAC-3’）は，*COI* 遺伝子に隣接し，保存性の高い *tRNA (Tyr)* 遺伝子領域の塩基配列に基づいて作成した．COI-b（リバースプライマー：5’-GGATAAAGGTGGATAiACiGTTCA-3’）は，DGGE 法を適用できる DNA 断片の限界長（約 500 塩基対）を考慮して，試験種 10 種を含むショウジョウバエ科 34 種の *COI* 遺伝子の塩基配列を比較して，COI-a から 354 塩基離れたところに，*D. melanogaster* の配列を基準として作成した（表 2）．*COI* 遺伝子のこの領域には変異サイトが多く含まれており，すべての種に共通する塩基配列のプライマーを設計することはできなかったので，特に 3’ 端側のミスマッチを最小限にするために，ユニバーサルヌクレオチドであるイノシン(i)を 2 ヵ所に挿入した．また，DGGE 法を適用するための PCR 増幅では，バンドの分離能を向上させるために，COI-a の 5’ 末端に GC クランプ（5’-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3’）を付加した．

2.3　実験 1

実験の流れを図 1 に示す．

2.3.1　ゲノム DNA の抽出と鋳型 DNA の調整

ゲノム DNA の抽出は，小林・舘（2009）に記載されている方法を用いて行った．

DNA 抽出は，二通りの組み合わせのサンプルから行い，それぞれ後述の解析に用いた．一つは，各種の供試成虫 1 個体から別々にゲノム DNA を抽出し，それぞれ

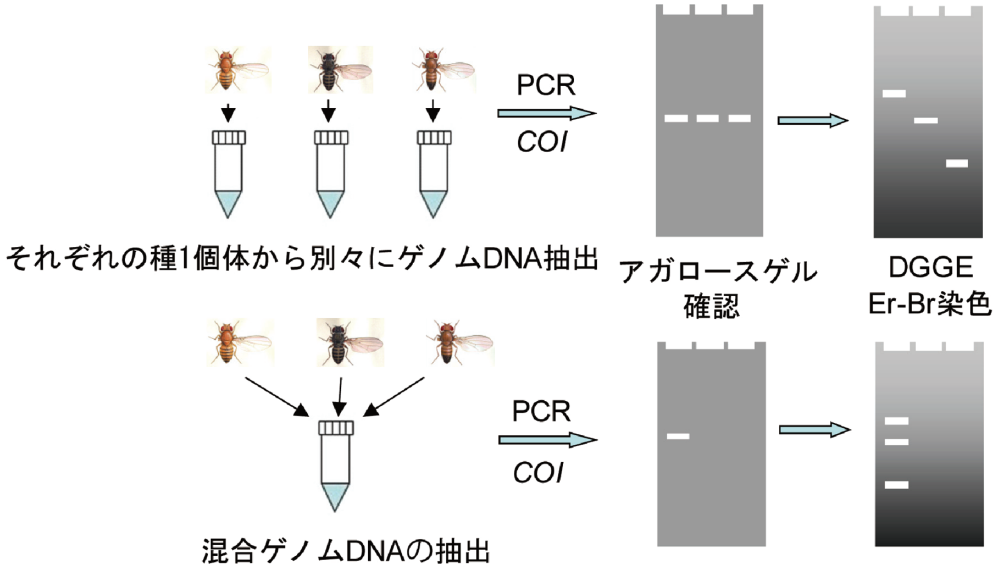

図 1：実験 1 の流れ．

表2：*COI* 遺伝子断片をPCR増幅するために設計したリバースプライマー（COI-b）の塩基配列とショウジョウバエ科34種の塩基配列の適合性

Primer (COI-b)	5'-	G	G	A	T	A	A	A	G	G	T	G	G	A	T	A	i	A	C	i	G	T	T	C	A	-3'
D. melanogaster*		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	**A**	•	•	•	•	•	
D. simulans		**A**	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	**A**	•	•	**A**	•	•	•	•	•	
D. yakuba		**A**	•	•	•	•	•	•	•	•	**A**	•	•	**G**	•	•	**A**	•	•	**A**	•	•	•	•	•	
D. pseudoobscura		T	•	•	•	•	G	T	•	•	•	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
D. bifasciata		T	•	•	•	•	G	C	•	•	•	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
D. willistoni		T	•	•	•	•	•	G	•	•	•	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
D. paulistorum		T	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
D. nebulosa		T	•	•	•	•	•	T	•	•	•	•	•	•	•	•	T	•	•	T	•	•	•	•	•	
D. saltans		A	•	•	•	•	•	G	•	•	A	•	•	•	•	•	A	•	•	T	•	•	•	•	•	
D. emerginata		•	•	•	•	•	•	•	•	•	A	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
L. collinella		A	•	•	•	•	•	G	•	•	C	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
L. stackelbergi		**T**	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	**A**	•	•	**G**	•	•	•	•	•	
L. clarofinis		A	•	•	•	•	G	G	•	•	•	•	•	G	•	•	A	•	•	G	•	•	•	•	•	
L. magnipectinata		A	•	•	•	•	•	•	•	•	A	•	•	G	•	•	A	•	•	G	•	•	•	•	•	
D. virilis		**T**	•	•	•	•	•	**T**	•	•	**A**	•	•	**G**	•	•	**A**	•	•	**T**	•	•	•	•	•	
D. robusta		T	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
D. sordidula		C	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
D. repleta		T	•	•	C	•	G	•	•	•	A	•	•	•	•	•	A	•	•	A	•	•	•	•	•	
D. hydei		**T**	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	**A**	•	•	**A**	•	•	•	•	•	
D. annulipes		T	•	•	•	•	•	•	•	•	A	•	•	•	•	•	A	•	•	T	•	•	•	•	•	
D. immigrans		**T**	•	•	•	•	•	•	•	•	**A**	•	•	**G**	•	•	**A**	•	•	**T**	•	•	•	•	•	
D. funebris		•	•	•	•	•	•	**T**	•	•	**A**	•	•	**G**	•	•	**A**	•	•	**A**	•	•	•	•	•	
D. maculinotata		A	•	•	•	•	•	•	•	•	A	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
D. palustris		A	•	•	•	•	•	C	•	•	G	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
D. guttifera		T	•	•	•	•	•	T	•	•	•	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
M. poecilogastra		A	•	•	•	•	G	T	•	•	G	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
H. macromaculata		A	•	•	•	•	•	T	•	•	G	•	•	G	•	•	C	•	•	G	•	•	•	•	•	
H. alboralis		A	•	•	•	•	•	C	•	•	A	•	•	G	•	•	A	•	•	T	•	•	•	•	•	
H. quadrivittata		A	•	•	•	•	•	•	•	•	•	•	•	G	•	•	A	•	•	A	•	•	•	•	•	
Di. tenuicauda		•	•	•	•	•	•	•	•	•	G	•	•	G	•	•	T	•	•	T	•	•	•	•	•	
Di. pseudotenuicauda		A	•	•	•	•	A	•	•	•	G	•	•	G	•	•	T	•	•	T	•	•	•	•	•	
Di. acutissima		T	•	•	•	•	•	•	•	•	A	•	•	•	•	•	A	•	•	A	•	•	•	•	•	
C. procnemis		**T**	•	•	•	•	**G**	**T**	•	•	**A**	•	•	•	•	•	**A**	•	•	**A**	•	•	•	•	•	
S. coracina		**T**	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	**A**	•	•	**T**	•	•	•	•	•	

*太字：供試種．

を〝単一種ゲノムDNA〞とした．もう一つは，10種の供試成虫をそれぞれ1個体ずつ選び，それらを混ぜたサンプルから抽出したDNAを〝混合ゲノムDNA〞とした．

また，混合ゲノムDNAは，鋳型DNA濃度の違いによるPCR増幅への影響を調べるために，濃度を$2^{-1\sim-8}$倍に希釈した試料を準備した．

2.3.2 PCR増幅

抽出したゲノムDNAを鋳型とし，作成したプライマーセット（GCクランプ+COI-aとCOI-b）を用いてPCR増幅を行った．PCRにはTaKaRa Ex Taq (TaKaRa) を用い，以下の組成の反応液中で行った：10×EX Taq buffer 2ul，0.2mM dNTP混合液1.6ul，10pM各プライマー0.4ul，5U/ul EX Taq polymerase 0.2ul，滅菌蒸留水14.9ul．PCRは，94℃で3分間初期熱変性させた後，94℃30秒・56.3℃30秒・72℃1分を30サイクル繰り返し，最後に72℃で8分間伸長反応させた．得られたPCR産物の一部を，1.5%アガロースゲルで電気泳動した．電気泳動後，ゲルをエチジウムブロマイドで染色し，トランスイルミネーター上でUV照射により目的の増幅産物を確認した．

2.3.3 DGGE解析

得られたPCR産物について，DCode system (Bio-Rad) を用い，石井ほか（2000）に解説されている方法に従って，以下のDGGE条件で塩基配列の違いによる分離を行った．6%ポリアクリルアミド（アクリルアミド：ビス=37.5：1）に，5%～25%の濃度勾配で変性剤（100%変性剤は7M尿素，40%ホルムアミドに相当）を加えたゲルを作成し，0.5×TAE緩衝液中で，60℃，150V定電圧下で5時間，電気泳動を行った．泳動終了後，ゲルをエチジブロマイドで染色し，トランスイルミネーター上でUV照射によってバンドを可視化し撮影を行った．

2.4 実験2

実験の概要を図2に示す．

実験1のDGGEで明瞭に分離したバンドをゲルから

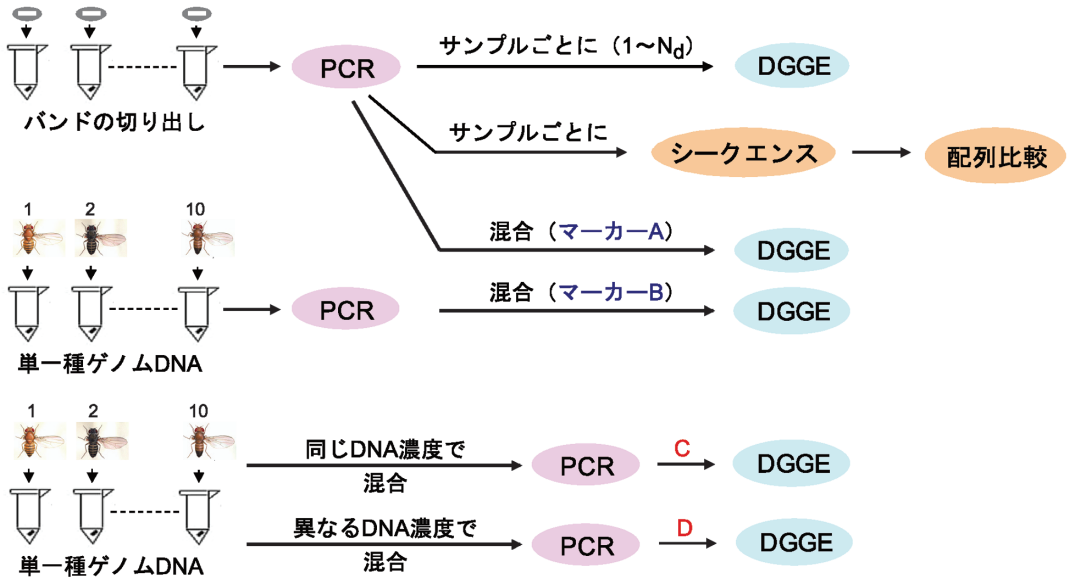

図 2：実験 2 の概要．

切り出し，それを鋳型 DNA として PCR の再増幅を行った．得られた再増幅産物の塩基配列を，それぞれ，DNA シーケンサー ABI PRISMTM 377 (Applied Biosystems) によって決定し，GenBank に登録されている，あるいは Katoh (1999) に報告されている既知の *COI* 塩基配列との相同性を比較した．

また，PCR 再増幅産物をそれぞれ，同じ DGGE 条件で泳動し，バンド位置の再現性を確認した．同時に，それらを混合した試料(A)と実験 1 の単一種ゲノム DNA から増幅された PCR 産物を混合した試料(B)を，それぞれマーカーとして泳動した．

さらに，混合ゲノム DNA から PCR 増幅を行う際に，各種の DNA 濃度の違いによる影響を調べるために，単一種ゲノム DNA をそれぞれ等濃度になるように調整して混合した鋳型 DNA(C)と濃度を調整せずに混合した鋳型 DNA(D)から PCR 増幅を行った．また，実験 1 の結果を再確認するために，同じ鋳型混合ゲノム DNA から再度 PCR 増幅し，これらの産物をそれぞれ上記の試料とともに DGGE 分析した．

3．結果と考察

3.1　実験 1

3.1.1　単一種ゲノム DNA からの PCR-DGGE

単一種ゲノム DNA から増幅した PCR 産物の DGGE 結果を，図 3 のレーン 1〜10 に示した．2 本のバンド（a と b）が検出されたレーン 3 の *D. virilis* を除いて，各レーンにおいて，それぞれ単一で明瞭なバンドが確認された．また，各バンドの出現位置は種ごとに異なっており，互いに区別できるように分離された．

3.1.2　混合ゲノム DNA からの PCR-DGGE

次に，10 種各 1 個体の供試虫を混合して抽出したゲノム DNA からの PCR-DGGE 結果を，図 3 のレーン N_0〜N_8 に示した．$N_{1\sim8}$ は，PCR に適切な鋳型 DNA 濃度を調べるために，抽出した混合ゲノム DNA を $2^{-1\sim-8}$ に希釈して行った PCR-DGGE の結果である．いずれの濃度においても，2 本の明瞭なバンド（c と d）しか得られなかった．

3.1.3　考察

各種の単一種ゲノム DNA からの PCR-DGGE は，それぞれ異なる位置に明瞭なバンドを形成した．つまり，DGGE 法によって種を判別することが可能であると期待される．この結果については，得られた各 DGGE バンドの位置に再現性があるかどうか，そして，出現した各バンドがそれぞれ実験に供した種由来の DNA 断片であるかどうかを，確認する必要がある．

混合ゲノム DNA からの PCR-DGGE では，明瞭なバ

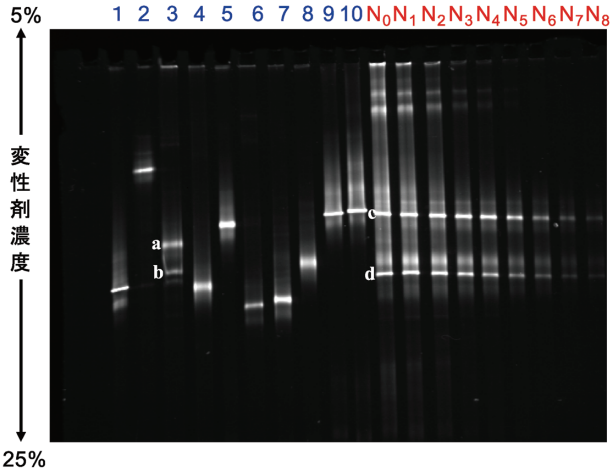

図 3：実験 1 による DGGE バンドパターン．1〜10 は単一種ゲノム DNA からの PCR 産物（番号は，表 1 の種 No. に対応），N_0 は混合ゲノム DNA からの PCR 産物，$N_{1\sim8}$ は混合ゲノム DNA を $2^{-1\sim-8}$ に希釈した鋳型 DNA からの PCR 産物．

ンドは2本しか出現しなかった．この原因としては，(1)鋳型混合ゲノムDNA中のDNA濃度の種間差と，(2)それぞれの種の *COI* 配列とプライマー配列との適合度の違いによる，PCR増幅におけるバイアスが考えられる．

3.2　実験2

3.2.1　DGGEバンドの再現性と塩基配列の確認

実験1で得られたDGGEゲルから，13のバンドを切り出してPCRによる再増幅を行い，DGGEで結果の再現性を確認した．その結果，それぞれのバンド位置は前回とほぼ同じであった（図4：レーン1，2，3_a，3_b，4–10，N_c，N_d）．また，各バンドから切り出したDNAの塩基配列は，それぞれの種の *COI* 遺伝子の既知の配列と非常に高い（99%以上）相同性を示した．*Drosophila virilis* で検出された2本のバンドのうち，3aは *D. virilis* の *COI* 配列と100%一致していたが，3bは9カ所の塩基に違いが認められた．混合ゲノムDNAから検出されたバンド N_c は *D. melanogaster* の配列と100%，バンド N_d は *S. coracina* の配列と99%一致した．

3.2.2　DGGEマーカーの作成

図4のレーンA，Bはそれぞれ10種の単一種鋳型DNAから得られたPCR産物の混合物だが，どちらも10（11）本のバンドで構成された明瞭なマーカーにはならなかった．

3.2.3　混合ゲノムDNAからのPCR-DGGE解析

図4のレーンC，D，N_o’は，いずれも混合ゲノムDNAを鋳型としたPCR-DGGEの結果であるが，実験1と同様に，どれも10（11）本の明瞭なバンドで構成されたDGGEバンドパターンは検出されなかった．ただし，10種それぞれの単一種ゲノムDNAを等濃度になるように調整して混合したCと濃度調整をせずに混合したDを比べると，Cの方でより多くのバンドが検出されている．また，実験1の N_o と比べて N_o’の方がバンドの検出がよいように見える．

3.2.4　考察

今回の実験で設定したDGGE条件は，同じDNA分子種に対して，そのバンド位置に関して非常に高い再現性を示した．また，今回 *COI* 遺伝子の部分配列に対して設計したプライマーは，実験に供した10種それぞれの単一種ゲノムDNAを鋳型にすれば，充分に目的とするDNA断片を増幅できることがわかった．ただし，*D. virilis* では，標的とした *COI* 断片の他に，配列が9塩基異なるパラログ断片も増幅した．

2種類のDGGEマーカー試料（AとB）を作成して泳動したが，バンドの解像度はあまりよくなかった．この点は，DGGE条件を調整することで改良できる．バンドが明瞭でなく影ができるのは，泳動時間が足りないからだと考えられる．また，一部のバンドが互いに非常に近い位置に現れる問題（例えば，レーン9と10など）は，ゲルの変性剤濃度勾配をさらに狭くすることで，バンドの分離を向上させることができるかもしれない．しかし，それは構成種の組み合わせによって異なり，今回の試験のように全構成種がわかっている場合には好適な条件を調整できるが，一般にどんな種が含まれているかわからないサンプルに適用する際には，部分的にバンドが重なる可能性があることは避け難い．

実験1，2とも，混合ゲノムDNAからのPCR-DGGEで，目的とした10種全ての明瞭なバンドを検出できなかった．その原因としては，まず，鋳型混合ゲノムDNAからPCR増幅を行う際の鋳型DNA中の

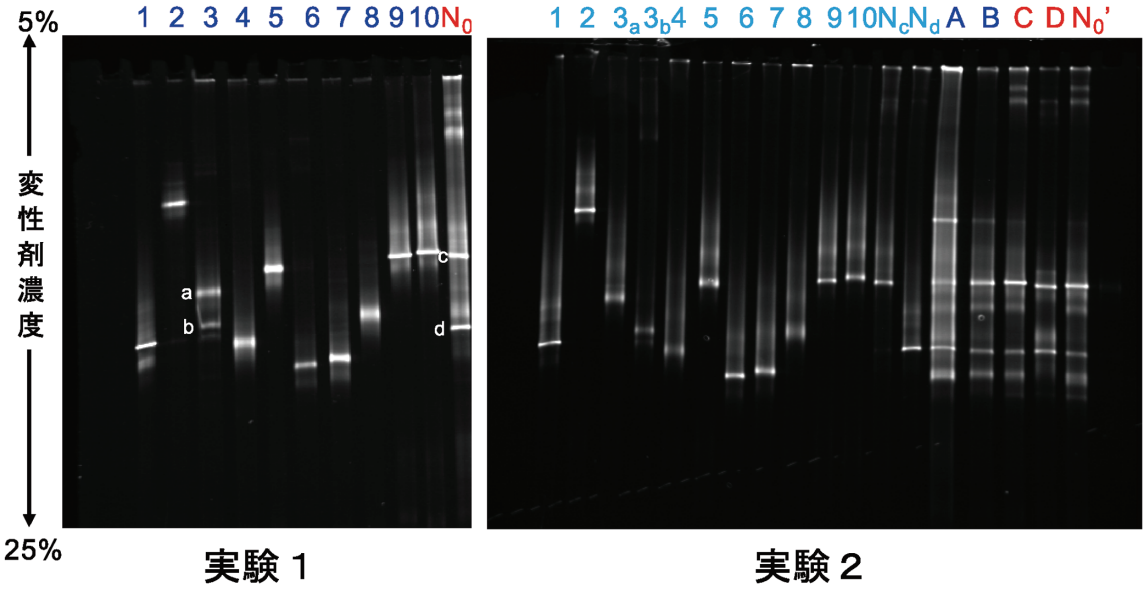

図4：実験2によるDGGEバンドパターン（実験1との比較）．1～N_d は実験1のゲルから各バンドを切り出して再増幅したPCR産物，CとDは単一種ゲノムDNAをそれぞれ等濃度になるように調整して混合した鋳型DNA(C)と濃度を調整せずに混合した鋳型DNA(D)からのPCR産物，N_0’は実験1の N_0 と同じ鋳型混合ゲノムDNAから再度PCR増幅した産物，AとBはマーカーレーン（説明は本文参照）．

DNA濃度の種間差によるバイアスが考えられる．実際に，10種それぞれの単一種ゲノムDNAを濃度が均一になるように混合した鋳型DNAから増幅したPCR産物を泳動したレーンCでは，濃度が不均一な場合のレーンDよりも多くのバンドが検出できた（図4）．このことは，PCR-DGGE法を一般の生物（昆虫）群集サンプルに適用する際に重要な問題があることを意味している．つまり，種間の相対頻度に差がある（自然界の生物群集）サンプルからは，PCR-DGGE法では優占種に正のバイアスがかかった結果が得られるということになる．微生物群集でも，群集の1％以上を占める種は検出できたという報告もあるが（Muyzer et al., 1993），この解像度はいろいろな条件によって変化することがわかっている（石井ほか，2000）．

次に，それぞれの種の*COI*配列とプライマー配列との適合度の違いによるPCRバイアスが考えられる．本研究では，リバースプライマー（COI-b）を試験に供した10種に共通した配列で設計できなかった（DGGE法を適用できるDNA断片長に制限があることにより，*COI*遺伝子の種間変異の多い配列部位に設定せざるを得なかった）．プライマー（COI-b）配列の基準とした*D. melanogaster*が最も適合度が高いのは当然として，次いで，*S. coracina*，*D. simulans*，*L. stackelbergi*の適合度が高かった（2塩基違い；表2）．これらのうち，*D. melanogaster*と*S. coracina*のバンドが，実験1，2における混合ゲノムDNAからのPCR-DGGEで（$N_{o\sim8}$，N_o’，C，D），それぞれ明瞭なバンドとして検出された．この結果から，プライマー配列との適合性はPCR増幅において非常に重要な影響を及ぼすと考えられる．実際に，プライマー配列との相同性が高いものほど優先的に増幅する傾向があることは，細菌を対象としたPCR-DGGEにおいて，重要な問題の一つとして指摘されてきた（例，Ishii and Fukui, 2001）．

また，まったく同じ混合ゲノムDNAを鋳型としたにもかかわらず，実験1（N_o）と実験2（N_o’）のPCR-DGGEの結果に差が出たことは，メタゲノムを鋳型とした場合，同じ条件下のPCR増幅でも産物に差が出る可能性があることを示している．

4．まとめと今後の展望

今回の実験で，PCR-DGGE法を昆虫群集サンプルに適用する場合，PCR増幅段階でのバイアスが問題であることが明らかになった．これは，群集構成種を全て検出しようとするなら，微生物群集を含めてどんな生物群集にも付きまとう問題である．プライマーの問題は，対象とする生物群でより保存性の高いシングルコピー遺伝子の配列部位を探索すれば，ある程度は解決できるだろう．しかし，完全なユニバーサルプライマーの設計は理論上無理である．また，本研究の*D. virilis*で見られたように，パラログも増幅してしまう可能性も排除できない（メタゲノム-PCR-DGGEでは，別のバンド，〝種〟，として検出され得る）．もっと根本的な問題は，鋳型メタゲノム中でのDNA濃度の種間差によるPCRバイアスである．つまり，PCR-DGGE法では，群集（サンプル）の中の相対頻度（≒DNA濃度）組成を正確には評価できないということである．さらに，一般に種構成が未知のサンプルに対して，ゲル上でのバンドの分離能を向上させるDGGE条件を決定できない（部分的なバンドの重なりの可能性を排除できない）という問題もある．

以上の問題を考慮すると，メタゲノムに対するPCR-DGGE法の適用範囲は，ある程度限られたものにならざるを得ない．例えば，構成種が既知のある範囲に限られた群集の種組成の解析・比較，あるいは，メタゲノムの中からPCR-DGGE法で検出できる構成種のみから成る分群集を対象とした研究などである．実際に，微生物群集のPCR-DGGE法による研究は後者の例ある．一般に多くの生物群集研究は，ある方法でスクリーニングされた分群集を対象にしている（例えば，ライトトラップで採集された昆虫群集などのように）のであるから，PCR-DGGE法でスクリーニングすることに本質的な違いはないかもしれない．しかし，本研究で目指した網羅的な生物多様性観測のための新しい手法の開発という点では，適用が難しいと言わざるを得ないであろう．

しかし，DNA塩基配列情報を使った他の方法も考えられる．PCR-DGGE法は，メタゲノムから共通プライマーによって構成種のDNA断片を増幅し，それらを塩基配列の違いによって分離するという方法であるが，メタゲノムから種特異的プライマーによってDNA断片を増幅してそれぞれの種の存否を確認するという方法も考えられる．これをリアルタイムPCRと組み合わせて行えば，鋳型メタゲノム中のそれぞれの種のDNA濃度も推定でき，群集の相対頻度組成も評価できる可能性がある；DNA濃度を相対頻度（個体数かバイオマスか）に換算する検証実験が必要であるが．この方法は，既に海洋プランクトンのメタゲノムから特定の1，2種の個体群密度を推定するのに使われており（例えば，Hosoi-Tanabe and Sako, 2005；松村ほか，2008），また，群集組成解析の試みも始まっている（首都大学東京の田村浩一郎氏からの私信）．しかし，この方法もPCR増幅過程を含むので，その段階でのバイアスが考えられる．まず，方法論上，存在が予想される（プライマーが設計された）種しか検出できない．PCR-DGGE法には発見的意義（未知種のバンドも検出される）があるが，この方法にはない．未知種（パラログも）の配列がどれかのプライマーと一致すれば，それが混ざって増幅されてしまう可能性もある．鋳型メタゲノムを小分けして種特異

的プライマーによるPCRを行うので，その段階での構成種DNA濃度組成のサンプリング誤差も生ずるかもしれない（稀少種は，PCR-DGGE法と同様に，検出されない可能性がある）．結局，この方法の適用も限られたものとならざるを得ないであろう．

理想の方法は，環境（サンプル）メタゲノムを直接配列決定して，構成種のゲノムコピー数をカウントする方法であろう．それが，次世代シークエンサーを使うことによって可能になり，既に，微生物群集の解析に適用され始めている（例えば，Qin et al., 2010）．これが生物多様性観測の実用的な方法となるためには，ゲノム（少なくとも標的DNAマーカー）配列のデータベースを整備し，次世代シークエンサーによる配列解読のコストが下がる必要があるが，分子生物学技術とバイオインフォマティクスの目覚ましい発展速度を考えると，そう遠くない将来には実現できるのではなかろうか．

このようなDNA塩基配列情報を使った生物種の同定方法は，従来の同定作業と異なり，一過性のものではなく，得られた配列情報がデータベースに蓄積すればするほどにその有用性が高まる性質のものである．また，この技術を消化管内容物や寄生者群集などに適用できれは，簡単に量的食物網のデータも得られるようになるなど，さまざまな可能性を秘めており，いわば「分子群集生態学」を拓く新技術となる可能性を有している．

しかし，専門的な分類学の知識を必要としない，だれにでもできる生物種の同定技術には，その長所の裏がえしとして，作業に携わる人の同定能力・知識の向上につながらないという欠点もある．また，DNA分子情報を使う場合には，証拠標本が残らないという問題点もある（証拠標本を残しつつ，DNAを抽出する方法もあるが，本研究で想定したような生物多様性観測で得られる膨大なサンプルの処理には適さない）．形態による識別や培養などが難しい多くの種を擁する微生物群集などの場合は仕方がないとしても，昆虫のように，形態による識別が比較的容易で，標本も残すことができる生物の場合は，従来の形態形質を使った分類・同定との併用が望ましい．ましてや，未知種の問題は，まずは専門の分類学者にその解明を急いでもらわなければならない（特に，生物相の解明が遅れている熱帯域で）．そのためには，発見的な意味を持っている分子生物学的手法（PCR-DGGE法やメタゲノム配列解読など）も役立つに違いない．

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ショウジョウバエの耐寒性と膜脂質

後藤　慎介[1)]

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ショウジョウバエの耐寒性の獲得にかかわる生化学的なメカニズムを探るため，キイロショウジョウバエの低温麻痺からの回復が早い系統（CR），遅い系統（CS），中間的な系統（CTL）を人為選択あるいは自由交配によって作製した．これらの系統から生体膜の流動性にかかわるリン脂質を抽出し，脂肪酸の組成をガスクロマトグラフィーによって調べたところ，系統間で大きな違いが見られた．耐寒性の獲得・喪失にこれらリン脂質脂肪酸の変化がどのように関わっているのかについて議論する．

Cold tolerance and membrane phospholipids in *Drosophila*

Shin G. Goto[1]

To clarify biochemical mechanisms underpinning acquisition of cold tolerance, I investigated the fatty acid compositions of phospholipids in *Drosophila melanogaster* lines showing rapid (CR), intermediate (CTL), or slow (CS) recovery from chill coma, which were established by artificial selection or by free recombination without selection. Compared to CTL, CS showed a low composition of dienoic acids and a small number of double bonds in the fatty acids. The ratio of unsaturated fatty acids and saturated fatty acids (UFAs/SFAs) was significantly lower in CS than in CTL. CR had higher monoenoic acid composition and lower dienoic acid composition than CTL. In addition, the amount of SFAs was lower and therefore the UFAs/SFAs ratio considerably higher in CR than in CTL. These changes in phospholipid fatty acids probably contributed to losing and maintaining the homeoviscosity of the cellular membranes in CS and CR, respectively, at low temperatures and therefore produced their distinct phenotypes in recovery from chill coma.

1．はじめに

冷え込む夜，一年の中で次第に寒くなる秋そしてさらに厳しい冬．温度の低下はたとえわずかであっても，外温動物である昆虫にとっては致命的になりうる．ショウジョウバエはどのようなしくみで寒さに耐えているのだろうか．非常にシンプルな問いかけであるが，これにきちんと答えることは難しい．これまでの研究から，糖や糖アルコールの蓄積，種々のストレスタンパク質の発現，水の管理など，複数のメカニズムが協調して働くことによって「寒さに強い」からだを作り上げていることが明らかになってきたが，その詳細はいまだに大きな謎に包まれている（Lee, 2010）．本稿では，耐寒性獲得のメカニズムの一つである膜脂質の質的変化に着目し，この変化がショウジョウバエの耐寒性の獲得にどのように貢献しているかについて議論する．

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2．生体膜の構造と温度

生体膜は外界との物理的な境界として働くとともに，外界との間で物質・情報・エネルギーの交換や変換を行う場としての役割を担う．生体膜の主成分は脂質とタンパク質であり，脂質は主にグリセロールを骨格とするグリセロリン脂質である（図 1A）．グリセロリン脂質ではグリセロールに 2 つの脂肪酸とリン酸がエステル結合しており，リン酸にはさらにエタノールアミン，コリンなどが結合する．エタノールアミンが結合したグリセロリン脂質をホスファチジルエタノールアミン（PE），コリンが結合したものをホスファチジルコリン（PC）と呼ぶ（図 1A）．哺乳類は PC が主体の膜を持つが，ショウジョウバエは PE 主体の膜を持ち，リン脂質の約 70％を PE が占める．リン脂質は両親媒性物質で，親水性の極性基を外側に，疎水性の非極性基（脂肪酸鎖）を内側に配列して二重膜を形成し（図 1B），この二重膜内

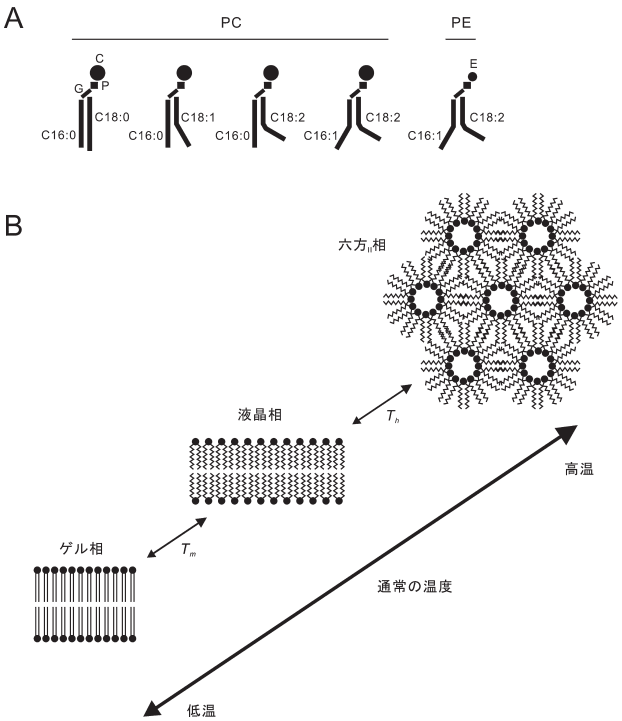

図1：(A)リン脂質の模式図．C：コリン，E：エタノールアミン，G：グリセロール，P：リン酸，PC：ホスファチジルコリン，PE：ホスファチジルエタノールアミン．脂肪酸はCx：yであらわす（xは脂肪酸の炭素数，yは二重結合の数）．飽和脂肪酸は直鎖であるが，不飽和脂肪酸は二重結合により折れ曲がる．(B)温度と相転移．通常の温度では脂質二重層は液晶相をとるが，低温条件下では相転移を起こしてゲル相となり，流動性が低下する．PE膜は高温条件下では六方$_{II}$相をとるために二重層が崩壊する．

にタンパク質分子がモザイク状に組み込まれている．この脂質二重層は流動性を持ち，膜中に組み込まれたタンパク質もある程度自由に移動することができる（流動モザイクモデル）．ここで強調したいのは，膜が「流動性を持っている」ということである．流動性があるからこそ，膜は情報・物質・エネルギーの伝達を行う場として働くことができる（Hochachka and Somero, 2002）．

しかし膜は温度に非常に敏感であり，温度の変化によってその状態は大きく変化する．図1Bに示すように，温度が低下すると液晶相を取っていた脂質二重層は相転移を起こし，ゲル相となって流動性が低下する（この転移温度をT_mという）．こうなると膜は膜としての機能を失ってしまう．言い換えるならば，生物が寒さに耐えるには低温下でも膜の流動性を維持する必要がある．PC主体の膜の相転移を考える際にはこのT_mに着目すればよいが，PE主体の膜はさらに複雑な相転移を起こす．PEはPCに比べて極性基が小さいため，全体として円錐形となる（図1A）．このようなリン脂質は高温になると極性基が集合した六方$_{II}$相をとり（この転移温度をT_hという），膜は崩壊してしまう（図1B）．すなわちPEが主体の膜は二つの相転移温度を持ち，その間でのみ液晶相をとることができる．PE，PCともに，相転移温度は脂肪酸の種類や組成によって変化する（Hochachka and Somero, 2002）．

二重結合を持たない脂肪酸を飽和脂肪酸（saturated fatty acid），二重結合をもつ脂肪酸を不飽和脂肪酸（unsaturated fatty acid）と呼ぶ．不飽和脂肪酸は二重結合によって約30°折れ曲がる（図1A）．このため，不飽和脂肪酸を持ったリン脂質は全体として円錐形となり，リン脂質同士が整列しづらくなる．これによって低温条件下においても膜はゲル化しづらくなる．低温に適応した昆虫では飽和脂肪酸に対する不飽和脂肪酸の割合（UFAs/SFAs）が高くなっている場合が多く（Koštál, 2010），低温下で膜の流動性を維持しようとしていることがうかがえる．

3．キイロショウジョウバエの人為選択

3.1 人為選択

北海道大学大学院地球環境科学院の森信人と木村正人は日本各地のキイロショウジョウバエ*Drosophila melangoaster*を集団で飼育し，人為選択によって温度麻痺からの回復時間が異なる系統を作製し，回復時間が他のどのような形質とリンクしているのかについて検討した（Mori and Kimura, 2008）．私たちはこの系統を譲り受け，人為選択を継続するとともに，低温に強くなるにはどのような生化学的・生理学的なしくみが重要なのか，について探ってきた（Goto et al., 2010; Udaka et al., 2010）．

まずこの人為選択の方法について説明する．羽化当日に麻酔なしで雌雄を分別し，飼育瓶に移して飼育する．羽化5日の成虫80－120個体を，0 °C 4時間の低温ストレスにさらす．すると，ハエたちは低温麻痺に陥り，微動だにしなくなる．その後25°Cに移すと，ハエたちは低温麻痺から回復して自身の脚で立ちあがる．25°Cに移してから立ちあがるまでの時間を測定し，上位10%の個体，下位10%の個体を選抜して次世代の親とした．このような選択を2 － 3世代毎に行った．以後，麻痺からの回復時間が早い系統をcold, rapidの略でCR系統，遅い系統をcold, slowの略でCS系統と呼ぶ．また，選択を行わなかった系統をコントロール（CTL）系統と呼ぶ．

人為選択によって，CRでは低温麻痺からの回復時間が早くなり，CSでは回復時間が遅くなった（図2）．このことは低温麻痺からの回復時間が遺伝的な制御を受けていることを示す．また，厳しい低温ストレス後の生存率を調べたところ，CRが最も低温に強かった（Udaka et al., 2010）．

では，これらの系統はどのようなリン脂質を持つのだろうか．

3.2 脂肪酸組成

本稿では脂肪酸をCx：y（xは脂肪酸の炭素数，yは

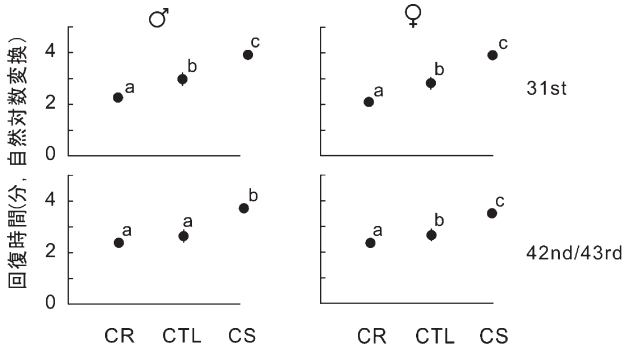

図2：人為選択系統とコントロール系統の低温麻痺からの回復時間．CR：低温麻痺からの回復時間が早い個体を選択した系統，CS：低温麻痺からの回復時間が遅い個体を選択した系統，CTL：コントロール系統．回復時間を自然対数変換して Tukey 検定にかけた．異なるアルファベットは統計的に有意な違いがあることを示す（$p<0.05$）．上段は 31 世代の個体の，下段は 42 あるいは 43 世代の個体のデータを表す．（Goto et al., 2010; Elsevier の許可を得て転載）

二重結合の数）と表記する．羽化 5 日のオスのリン脂質を抽出し，その脂肪酸を調べたところ，主な脂肪酸は C 16：0（パルミチン酸），C 16：1（パルミトレイン酸），C 18：0（ステアリン酸），C 18：1（オレイン酸），C 18：2（リノール酸），C 18：3（リノレン酸）であった．

CS 系統と CTL 系統の脂肪酸組成にはほとんど違いが見られなかったが，二重結合を二つ持つ脂肪酸（ジエン）の割合が CS 系統で有意に低くなっていた（図 3）．そのため，UFAs/SFAs の値も低くなっていた．さらに，脂肪酸中の二重結合数（＝∑(n 個の二重結合をもつ脂肪酸%)･n）を計算したところ，これも CS 系統で有意に低くなっていた（図 4）．二重結合数が少なく飽和脂肪酸に富むリン脂質からなる膜ではリン脂質が整列しやすく，低温環境下ではよりゲル相を取りやすくなる．低温麻痺からの回復に時間のかかる CS 系統は，確かに，低温環境下で流動性を維持するには不都合な膜を持っているようだ．

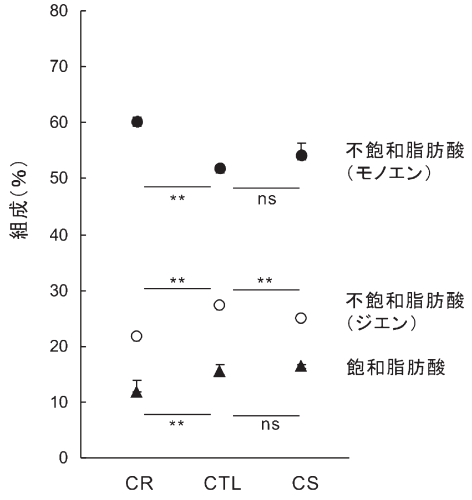

図3：人為選択系統とコントロール系統のリン脂質脂肪酸組成．中央値と四分位範囲で示す．CR：低温麻痺からの回復時間が早い個体を選択した系統，CS：低温麻痺からの回復時間が遅い個体を選択した系統，CTL：コントロール系統．脂肪酸組成を逆正弦変換し，Dunnet 検定で CTL との違いを検定した．**$p<0.01$；ns，$p\geq0.05$．（Goto et al., 2010; Elsevier の許可を得て転載）

CR 系統と CTL 系統を比較すると，CR 系統では C 16：0，C 18：2 の割合が低く，C 16：1，C 18：1 の割合が高かった．これにより CR 系統では飽和脂肪酸の割合が低く，二重結合が一つの脂肪酸（モノエン）の割合が高く，ジエンの割合が低かった（図 3）．特に，飽和脂肪酸の低下により UFAs/SFAs は著しく高くなっている（図 4）．これは低温環境下で膜の流動性を維持するには都合がよい．ではモノエンの割合の増加，ジエンの割合の減少は何を意味するのだろうか．

ショウジョウバエが PE 主体の膜を持つことを思い出してほしい．PE 主体の膜は他のリン脂質主体の膜とは異なり，いたずらにジエンの割合を増やすと液晶相と六方$_{II}$ 相の相転移温度（T_h）が低温側にシフトしてしまい，穏やかな温度であっても容易に膜が崩壊することが起こりうる．実際，PE からなる人工膜の結果をみると，C 18：2 を二つ持つリン脂質の場合には T_m が −40°C，T_h が −15°C となる．一方 C 18：1 を二つ持つ場合には T_m と T_h はそれぞれ −16°C と 10°C，C 16：1 を二つ持つ場合には −30°C と 42.5°C となる（Lewis et al., 1989）．このことからも，PE 主体の膜を持つショウジョウバエにおいてはジエンの割合を低下させてモノエンの割合を増加させることは，有益だと考えられる．

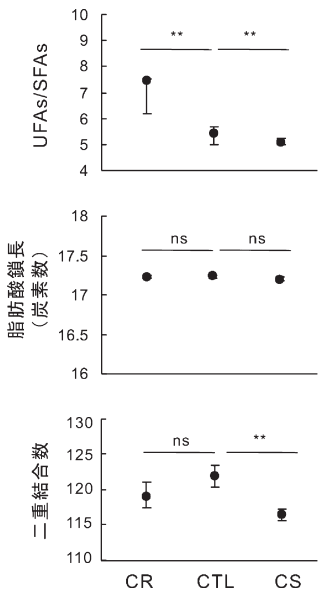

図4：人為選択系統とコントロール系統のリン脂質脂肪酸における飽和脂肪酸に対する不飽和脂肪酸の割合（UFAs/SFAs），脂肪酸鎖長，二重結合数．中央値と四分位範囲で示す．脂肪酸鎖長，二重結合数は以下のように計算した．脂肪酸鎖長＝[16･(炭素数 16 の脂肪酸%)＋18･(炭素数 18 の脂肪酸%)]/100，二重結合数＝∑(n 個の二重結合をもつ脂肪酸%)･n．Dunnet 検定で CTL との違いを検定した．**$p<0.01$；ns，$p\geq0.05$．（Goto et al., 2010; Elsevier の許可を得て転載）

4．キイロショウジョウバエ種群の種での知見

Ohtsu et al. (1998) は，キイロショウジョウバエ種群に属する亜熱帯性，暖温帯性，冷温帯性ショウジョウバエの 6 種 7 系統を短日，長日あるいは低温条件下で飼育し，その耐寒性とリン脂質脂肪酸組成を調べた．その結果，図 5 に示すように，低温に強いグループほど飽和脂肪酸の割合が低く，モノエンの割合が高く，ジエンの割合が低いことがわかった．この結果は上で述べた CR 系統の結果と同じである．人為選択によって耐寒性を獲得したキイロショウジョウバエ，自然選択によって耐寒性を獲得したショウジョウバエが，同じような脂肪酸組成の変化を示すことから，「飽和脂肪酸の割合の低下，モノエンの割合の上昇，ジエンの割合の低下」はショウジョウバエの耐寒性の獲得において非常に重要な役割を果たすと考えられる．

Ohtsu et al. (1998) は上記の結果に加えて，寒さに強いショウジョウバエでは炭素数 16 の脂肪酸の割合が高く，炭素数 18 の割合が低くなることを明らかにした．リン脂質中の脂肪酸が短い場合 T_m は低くなるので，このような変化は低温下で膜の流動性を保つには有益である．しかし，このような脂肪酸鎖長の変化は人為選択によって耐寒性を獲得したキイロショウジョウバエでは見られなかった（図 4）．より厳しい低温ストレスでの選択を行えば脂肪酸鎖長の変化も生じたのだろうか，そもそも低温にかなり弱いキイロショウジョウバエが耐寒性を獲得するうえではこれが限界なのだろうか．あるいは脂肪酸鎖長の変化は耐寒性の獲得には直接関係していないのだろうか．興味つきないもののその詳細は不明である．

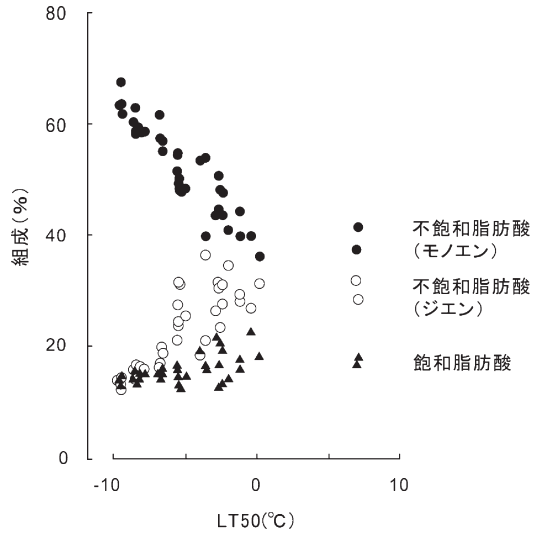

図 5：キイロショウジョウバエ種群のショウジョウバエ（*Drosophila subauraria*, *Drosophila biauraria*, *Drosophila triauraria*, *Drosophila rufa*, *Drosophila lutescens*, *Drosophila takahashii*）を 24 時間の低温にさらした際の半数致死温度（LT50）と不飽和脂肪酸（モノエン，ジエン）・飽和脂肪酸の割合．低温に強いショウジョウバエほど，飽和脂肪酸の割合が低く，モノエンの割合が高く，ジエンの割合が低い．（Ohtsu et al., 1998; Elsevier の許可を得て転載）

5．今後の課題

上述のキイロショウジョウバエの人為選択実験，そして Ohtsu らによるキイロショウジョウバエ種群の種での実験では，リン脂質の抽出後に脂肪酸だけを取り外してその分子種を同定している．しかしこのような方法では，実際にどのような脂肪酸がひとつのリン脂質を構成しているのかがわからない．HPLC と ESI-MS（エレクトロスプレーイオン化質量分析）を組み合わせた手法によって，リン脂質の分子種自体を明らかにすることが可能であるので（Koštál et al., 2003; Overgaard et al., 2008），このような手法を取り入れた解析が必須である．さらには，突然変異体や遺伝学的な操作が可能なキイロショウジョウバエの利点を生かし，膜の脂肪酸組成あるいは極性基の割合を変化させることによって，耐寒性がどのように変化するのかを具体的に調べていくことで，耐寒性の獲得を担う生理学的なメカニズムを明らかにしていきたい．

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ハシリショウジョウバエにおける遺伝子組換え系統の確立

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ハシリショウジョウバエの一種，*Chymomyza costata* は北半球の冷温帯と亜寒帯に広く分布し，ショウジョウバエ科の中では珍しく，幼虫で休眠し越冬する．幼虫休眠の誘導には短日が有効で，札幌産では臨界日長が 14 時間付近にある．臨界日長はどのように計られるのだろうか？「光周反応の測時機構に概日時計が関わっている」という Bünning (1936) の仮説があり，この証明を目指して行った *C. costata* における遺伝子組換え実験の概要を紹介する．ターゲットにした遺伝子は *timeless* で，*piggyBac* と呼ばれるトランスポゾン由来の遺伝子組換えベクターを介して，*timeless* の強制発現系統やノックアウト系統を確立した．

Establishment of transgenic lines in *Chymomyza costata*

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A drosophilid fly, *Chymomyza costata* is living in the cool temperate and subarctic zones of the Northern Hemisphere. This species enters winter diapause at the larval stage in contrast to most of other drosophilid species. Short-day photoperiods are effective for the induction of larval diapause. The critical photoperiod of a wild-type strain collected in Sapporo is about 14 hours. How is the length of day or night measured? Bünning (1936) has proposed a hypothesis that the photoperiodic time measurement system involves the circadian clock. To test this hypothesis, I prepared two transgenic lines of *C. costata*. In these lines, the expression of a circadian clock gene, *timeless*, was enhanced or inhibited with heat shock treatment. The germline transformation was mediated by a lepidopteran transposon, *piggyBac*.

1. はじめに

マエグロハシリショウジョウバエ *Chymomyza costata* (Zetterstedt, 1838) は，ショウジョウバエ科，ハシリショウジョウバエ属の昆虫で，北半球の冷温帯と亜寒帯に広く分布する．分布域は北極圏にも及び，寒極の町として知られる東シベリアのベルホヤンスクでも採集されている．寒冷地域に生息するショウジョウバエ科の多くの種が成虫で越冬するのに対して，このハシリショウジョウバエは 3 齢（終齢）幼虫で休眠し，越冬する．

本種の幼虫休眠は，光周反応によって誘導され，短日が休眠の誘導に有効である．分布域の南限に近い札幌（北緯 43 度）産の個体群の臨界日長は，15°Cで飼育した場合，14 時間付近にある（Riihimaa and Kimura, 1989）．臨界日長は緯度が高くなる程，長くなる傾向があり，フィンランドのヴァルカウス（Varkaus，北緯 62 度）産では 19 時間 30 分である（Riihimaa and Kimura, 1989）．一方，光周反応を示さない非休眠系統も知られており，これは，北海道大学苫小牧研究林（北緯 42 度 40 分）で採集されたハエの子孫から交配実験によって選抜された（Riihimaa and Kimura, 1988）．

C. costata における臨界日長の地理的変異や光周反応を示さない系統の存在は，昆虫の光周反応を分子生物学的に解明するために重要である．私はチェコの昆虫学研究所の Kostal らとの共同研究によって，光周反応を示す系統と示さない系統における概日時計遺伝子の発現状態を比較し，光周反応を示さない非休眠系統では，本来，昼夜で振幅する *period* の発現が弱くなっていることを見出した（Kostal and Shimada, 2001）．さらに，光周反応を示さない系統では，*timeless* の発現が全く見られないことを突き止めた（Pavelka et al., 2003; Steh-

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lik et al., 2008)．こうした事実は，光周反応，特に昼あるいは夜の長さを計る測時機構に概日時計が関わっているというBünningの仮説を支持しているように見える．

しかし，概日時計遺伝子が光周反応に関わっていることを，もっと強力に証明するためには，光周反応を示さない系統で*timeless*を強制的に発現させて休眠性を回復させてみる必要がある．また，正常に光周反応を示す系統で*timeless*の発現をノックアウトして休眠性が失われることを確かめてみる必要がある．本報告では，こうした目的のためにトランスポゾン由来の遺伝子組換え用ベクターを用いて，*timeless*の強制発現系統とノックアウト系統を確立した方法を紹介する．

2．材料と方法

2.1 遺伝子組換え実験に用いたショウジョウバエ

実験に用いたショウジョウバエは，札幌産（野生型）の*C. costata*と苫小牧産の同種の子孫から分離された光周反応を示さない非休眠系統である．札幌産のハエは18°C16時間明期の長日条件で，非休眠系統は18°C10時間明期の短日条件で，ともに，ドライイースト，コーンミール，麦芽を含む寒天培地で継代飼育した．

2.2 遺伝子組換え用ベクターの作成

目的の遺伝子を強制発現あるいはノックアウトするための遺伝子組換え用のベクターは，pBac{3xP3-EGFPafm} (Horn et al., 2000)を改変することによって構築した．このプラスミド型のベクターは，鱗翅目昆虫のイラクサギンウワバ*Trichoplusia ni*のトランスポゾンである*piggyBac*に，緑色蛍光蛋白質（GFP）のDNA配列を乗せ，pUC18に組込んだものである．緑色蛍光蛋白質は，遺伝子組換えの成否を可視化するマーカーとして組込まれ，その発現は，3xP3プロモーターに眼の発生に関わる転写因子のPax-6/eyelessが結合することによって促進される．pBac{3xP3-EGFPafm}にはマルチクローニングサイトがあり，制限酵素の*Asc*I，*Fse*I，*Bgl*IIによって切断される．この部位に目的のDNA配列を挿入することができる．

*timeless*遺伝子を強制発現させるためのベクター，pBac{EGFP-tim}は，pBac{3xP3-EGFPafm}の*Asc*Iサイトに*C. costata*の*timeless* cDNAをヒートショック蛋白質，*hsp70*のプロモーターとターミネーター（polyA付加配列）の間に挟んで挿入したものである．まず，*timeless* cDNA (GenBank accession No. AB073724)の塩基配列番号452–4522の翻訳領域を逆転写PCR (RT-PCR)法によって増幅した．鋳型には*C. costata*の成虫頭部から抽出した総RNAを用いた．プライマーの配列は，timF（5’-AAGCGGCCGCATGGACTGGTTACTCGCAAC-3’）（センス配列）とtimR（5’-AAGCGGCCGCTCAATGATAGTAGGGCACAC-3’）（アンチセンス配列）で，ともに5’側に*Not*Iで切断される配列が付加されている．PCR産物をWizard SV Gel and PCR Clean-Up System (Promega)で精製後，*Not*Iで処理した．つぎに，*hsp70*のプロモーターとターミネーターの配列を持つP因子ベクター，pP{CaSpeR-hs}のマルチクローニングサイトを制限酵素の*Not*Iで切断し，開いた部位に上記のPCR産物をT4リガーゼを用いて連結した．*timeless* cDNAの配列を含むpP{CaSpeR-hs}をDH5αコンピテントセルに取込み，LB寒天培地で培養した．増殖したpP{CaSpeR-hs}をアルカリSDS法で抽出・精製し，これを鋳型にして，*timeless* cDNAを組込んだ*hsp70*プロモーターからターミネーターまでの部分をPCRで増幅した．プライマーの配列は，hspF (5’-AAGGCGCGCCATCCCAAAACAAACTGGTTA-3’)とhspR (5’-AAGGCGCGCCTCCTGACCGTCCATCGCAAT-3’)で，ともに5’側に*Asc*Iで切断される配列が付加されている．このPCR産物を前述のPromegaのキットで精製し，*Asc*Iで処理後，同じく*Asc*Iで処理されたpBac{3xP3-EGFPafm}に連結した．こうして構築されたpBac{EGFP-tim}をDH5αに取込んで増殖し，Plasmid Mini Kit (Qiagen)で精製後，遺伝子組換え実験に用いた．

*timeless*遺伝子をノックアウトするためのベクター，pBac{EGFP-timIR}は，pBac{EGFP-tim}の*timeless* cDNA翻訳配列を逆向き反復配列に置換えたものである．まず，*timeless* cDNA (GenBank accession No. AB073724)の塩基配列番号883–1214の翻訳領域を，前述の総RNAを鋳型にして，逆転写PCR法で増幅した．プライマーの配列は，timIRF (5’-AAGAATTCTCGGAGACCAAGCTATCACC-3’)とtimIRR (5’-AAGGCCGGCCGACAATGACGCCTCAAACC-3’)で，それぞれ，5’側に*Eco*RIあるいは*Fse*Iで切断される配列が付加されている．PCR産物を精製後，*Eco*RIで処理し，消化物同士をリガーゼで連結して逆向き反復配列を作成した．つぎに，pP{CaSpeR-hs}の*hsp70*のプロモーターとターミネーターの配列を別々にPCR法で増幅した．プロモーター用のプライマーはhspF（前述）とhspPR (5’-AAGGCCGGCCTATTCAGAGTTCTCTTCTTG-3’)で，それぞれ，5’側に*Asc*Iあるいは*Fse*Iで切断される配列が付加されている．ターミネーター用のプライマーはhspTF (5’-AAGGCCGGCCGTCGACTAAGGCCAAAGAGT-3’)とhspTR (5’-AAAGATCTTCCTGACCGTCCATCGCAAT-3’)で，それぞれ，5’側に*Fse*Iあるいは*Bgl*IIで切断される配列が付加されている．ふたつのPCR産物を*Fse*Iで処理しリガーゼで連結した後，*Asc*Iおよび*Bgl*IIで処理した．消化物を同様に制限酵素処理されたpBac{3xP3-EGFPafm}に組

込み，DH5α に取込んで増殖した．増殖したベクターを精製後 *Fse*I で処理し，同じ制限酵素で処理した逆向き反復配列と連結した．こうして作成された pBac{EGFP-timIR}は SURE コンピテントセルに取込んで増殖し，Qiagen のキットで精製後，組換え実験に用いた．

2.3　遺伝子組換え用ベクターの注入

遺伝子組換え用ベクター，pBac{EGFP-tim}あるいは pBac{EGFP-timIR}と，転移酵素発現用のベクター（ヘルパーベクター），phspBac (Handler and Harrell, 1999) は，ともに注入緩衝液（5 mM KCl，0.1 mM NaH_2PO_4，pH 6.8）に溶解し，マイクロピペットを用いて，*C. costata* の初期胚に注入した．強制発現用の pBac{EGFP-tim}は光周反応を失った非休眠系統に，ノックアウト用の pBac{EGFP-timIR}は光周反応を示す札幌産（野生型）の初期胚に注入した．初期胚は産卵後 1 時間以内に採取した胞胚期以前の胚を使用した．胚をカバーグラス（5×18 mm）に貼付けた両面テープ上に整列し，70%エタノールに 1 分間浸けて除菌した後，実体顕微鏡のステージに固定した．マイクロピペットは外径 1 mm のガラスキャピラリー（GD-1, Narishige）をマイクロピペットプーラー（PC-10, Narishige）で引いて作成した．遺伝子組換え用ベクター（200 ng/μL）と転移酵素発現用ベクター（100 ng/μL）の混合液 2 μL をマイクロピペットに充塡し，マニピュレーター（MN-153, Narishige）に固定した．マイクロピペットを流動パラフィンを充塡したインジェクター（IM-6, Narishige）につなぎ，顕微鏡のステージとマニピュレーターを操作しながら，初期胚の後端に遺伝子組換え用混合液（10 nL）を注入した．初期胚の後端には，極細胞と呼ばれ後に生殖細胞に分化する細胞群がある．ショウジョウバエの遺伝子組換え体は，この極細胞の核 DNA に外来の遺伝子を組込むことによって作成される．

2.4　遺伝子組換え体のスクリーニング

遺伝子組換えベクターを注入された初期胚は，両面テープに貼付けたまま小型シャーレに移し寒天培地上で 25℃で培養した．注入から 1 日後，初期胚を 38℃で 30 分間熱処理して，転移酵素発現用ベクターを活性化し遺伝子組換えを促進した．孵化した幼虫はガラス管瓶（外径 3 cm，高さ 10 cm）に移し成虫まで寒天培地で飼育した．羽化した成虫（G 0 世代）同士を雌雄 1 個体ずつ交配し次世代（G 1 世代）を得た．G 1 世代の 2 齢および 3 齢幼虫を飼育瓶のガラス越しに簡易蛍光実体顕微鏡で観察し，中枢神経系から緑色蛍光を発する個体を選別した．簡易蛍光実体顕微鏡は，青色発光ダイオード（NSPB500S，日亜化学）を光源に使い，古い実体顕微鏡の対物レンズに UV フィルターとしてサングラスのレンズを被せた簡便なものである．選別した個体に由来する雌雄を継代飼育して組換え遺伝子がホモ接合（homozygous）である系統を確立した．

2.5　組換え遺伝子の発現確認

確立された遺伝子組換え系統における遺伝子の発現は，逆転写 PCR (RT-PCR) 法によって作成した cDNA の配列を解析して確認した．蛹化間近な 3 齢幼虫を 38℃で 30 分間熱処理した後，中枢神経系を含む前端部分を 10 個体集め，RNeasy (Qiagen) で総 RNA を抽出した．この RNA を鋳型にして，ThermoScript RT-PCR System (Invitrogen) で cDNA を作成した．プライマーには，Qt (5'-CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTT-3') を使用した．作成した cDNA を鋳型にして，*timeless* の組換え遺伝子を PCR 法で増幅した．プライマーには前述の timF と Qt および Qo (5'-CCAGTGAGCAGAGTGACG-3') を使用した．組換え遺伝子 *timeless* の後端部の DNA 配列を BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) で増幅した．プライマーには timSF (5'-GCAGTCAGTTCCAAGCAGGCC-3') を用いた．配列の決定には，ABI PRISM 3100-Avant Genetic Analyzer を利用した．

3．結果と考察

マエグロハシリショウジョウバエ *C. costata* を使った遺伝子組換え実験の組換え効率等を表 1 に示す．pBac{EGFP-tim}と pBac{EGFP-timIR}を構築するために

表 1：マエグロハシリショウジョウバエにおける遺伝子組換え効率

ベクター（Kbp）	初期胚	幼虫（%）	蛹（%）	成虫（%）	G 1 組換え体（%）	組換え系統（%）	世代数*
pBac{EGFP}**（6.5）	800	152（19.0）	128（16.0）	68（8.5）	15（1.875）	1（0.125）	42
pBac{EGFP-tim}（11.5）	954	174（18.2）		84（8.8）	1（0.1）	1（0.1）	30
pBac{EGFP-timIR}（8.2）	1320	146（11.1）		78（5.9）	3（0.23）	2（0.15）	16

*2010 年 12 月現在の世代数
**pBac{3xP3-EGFPafm}の略

用いた元ベクター，pBac{3xP3-EGFPafm}を初期胚に注入した場合，800 卵の胚から 152 個体（19.0%）の幼虫が孵化した．しかし成虫に羽化したのは 68 個体（8.5%）であった．羽化した雌雄（G 0 世代）を 1 個体ずつ交配して得られた G 1 世代の幼虫を蛍光実体顕微鏡で観察して，中枢神経系から緑色蛍光を発する個体を選別した結果，15 個体（1.875%）の遺伝子組換え体が得られた．鱗翅目昆虫のトランスポゾン，*piggyBac* を組込んだプラスミド型ベクターは，*C. costata* の遺伝子組換えにも有効であった．キイロショウジョウバエに由来する 3xP3 プロモーターも有効に働いて，緑色蛍光蛋白質を発現させた．しかし，このハシリショウジョウバエは，白眼のような，成虫でも緑色蛍光を識別できる系統を持たないので，遺伝子組換えのスクリーニングは幼虫でしか行えない．飼育瓶のガラス越しのスクリーニングでは見逃される組換え体もある．15 個体の遺伝子組換え幼虫に由来する雌雄を 1 個体ずつ交配し，これを数世代繰り返して，ホモ接合な系統を確立した．この過程で，産卵数の少ない雌雄は除かれたので，確立された系統はひとつだけであった．

timeless cDNA の翻訳領域や逆向き反復配列を pBac{3xP3-EGFPafm}に組込んで組換え実験を行った場合，幼虫の孵化率（18.2，11.1%）や成虫の羽化率（8.8，5.9%）に大きな変化はなかったが，組換え率（0.1，0.23%）は 1/10 近く減少した．組換えベクターの DNA サイズが大きくなる程，組換えが難しくなるものと思われる．結局，ホモ接合な組換え系統は，pBac{EGFP-tim}で 1 系統，pBac{EGFP-timIR}で 2 系統確立された．

pBac{EGFP-tim}を介して *timeless* の翻訳領域の cDNA が組込まれた，光周反応を示さない非休眠系統の幼虫では，38°C30 分の熱処理後，*timeless* mRNA の発現が見られた．図 1 に RT-PCR 産物をアガロース上で電気泳動した結果を示す．組換え系統を熱処理した場合，4Kbp 付近に *timeless* に由来すると思われる PCR

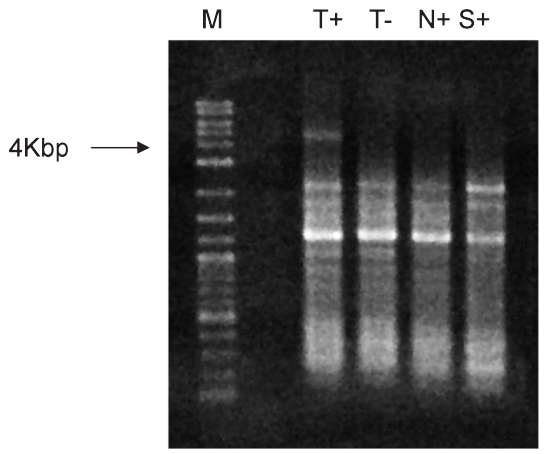

図 1：マエグロハシリショウジョウバエの遺伝子組換え幼虫における *timeless* 遺伝子の強制発現．図は，逆転写 PCR (RT-PCR) 産物のアガロース電気泳動図．M, 2-Log DNA Ladder (New England Biolabs)，矢印は 4Kbp を示す；T, pBac{EGFP-tim}による遺伝子組換え系統，＋は熱処理個体，－は非熱処理；N, 光周反応を示さない非休眠系統；S, 光周反応を示す札幌産野生型．

産物が確認できた．熱処理しなかった場合や非組換え系統では，*timeless* の発現は確認できなかった．キイロショウジョウバエ由来の *hsp70* のプロモーターはマエグロハシリショウジョウバエでも有効であった．

熱処理によって強制発現された *timelss* mRNA (cDNA) の配列は，図 2 に示すように，翻訳領域後端に続く非翻訳領域が *hsp70* のターミネーターの配列になっており，遺伝子組換えの事実がさらに確認できた．

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>tim_transgenic
CGGCATCTGTATACGAAAACGAGATGGCCAACTGCGACAATATATCGATGGCTTCGGAT
CTAGAGCATATGTATGTCAGTGATGAGGAGGACACACATGAGATACATGAGCAGCGTGT
GCCCTACTATCATTGAGCGGCCGCGGTCTAGAAGGCCTAATTCCAGCTGAGCGCCGGTC
GCTACCATTACCAGTTGGTCTGGTGTCGGGGATCCGTCGACTAAGGCCAAAGAGTCTAA
TTTTTGTTCATCAATGGGTTATAACATATGGGTTATATTATAAGTTTGTTTTAAGTTTT
TGAGACTGATAAGAATGTTTCGATCGAATATTCCATAGAACAACAATAGTATTACCTAA
TTACCAAGTCTTAATTTAGCAAAAATGTTATTGCTTATAGAAAAAATAAATTATTTATT
TGAAATTTAAAAAAAAAAAAAAAA
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図 2：遺伝子組換え系統において強制発現させた *timeless* mRNA (cDNA) の塩基配列．緑色配列は，*timeless* 翻訳領域の後端．黒色配列は非翻訳領域で，pBac{EGFP-tim}中の *hsp70* ターミネーターの配列になっている．

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ショウジョウバエにおける抗菌ペプチドの分子進化

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抗菌ペプチドはショウジョウバエにおいて主要な生体防御機構の一つであり，キイロショウジョウバエでは，これまでに 7 種類が同定されている．これら抗菌ペプチドは種類によって活性を示す対象の微生物が異なり，バクテリアに対して強い抗菌活性を示すものや真菌類に強い抗菌活性を示すものが知られている．様々な研究から，抗菌ペプチドは，個体レベルでは個体の微生物に対する抵抗性に大きく影響することが，遺伝子レベルでは種類によってその分子進化パターンが異なることが明らかとなっている．そこで，本稿では抗菌ペプチドの分子進化と抗菌活性や個体での抵抗性との関わりについて論じる．

Molecular evolution of antimicrobial peptides in *Drosophila*

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Antimicrobial peptides (AMPs) are essential components for *Drosophila* innate immune systems against microorganisms. Seven antimicrobial peptides have been identified in *D. melanogaster*, in which some AMPs show strong anti-bacterial activity, but others show anti-fungal activity. Repertoire of AMPs and their expression patterns are important for individual fly to survive under infection of Gram-negative, Gram-positive bacteria and fungi. Many molecular evolutionary analyses show different AMP genes have evolved in different manner. Cecropin and Drosomycin genes, for example, have evolved under frequent gene duplication and deletion event, so-called birth-and-death evolution, while Metchnikwin and Drosocin genes have kept only one copy in their host genome through *Drosophila* evolution. Ecologically, *Drosophila* is distributed among various environments from Tropical to Polar Zone, and some species have more resistance to bacteria or fungi than others have. However, the molecular-based mechanisms which affect a resistance to microorganisms still have not been elucidated clearly. In this paper, we introduce functions of *Drosophila* AMPs and their molecular evolution, and then discuss evolutionary relationship between them.

1. はじめに

抗菌ペプチドは自然免疫の主要な構成要素であり，原核生物から真核生物にわたる広範囲の生物が持つ免疫機構である．免疫機構の大切な機能は微生物を体内に侵入させないことであり，ショウジョウバエなどの昆虫では，クチクラでできた固い殻で体全体を覆うことで微生物の侵入を防いでいる．しかし，外殻に傷がついたり，餌と共に摂食することによって微生物が体内に侵入すると，ショウジョウバエ体内で様々な免疫反応が引き起こされる．免疫反応は細胞性免疫と体液性免疫反応に大きく分けられ，侵入した微生物は第一に，血球細胞による食作用（phagosytosis）や封入（encapsulation）などの細胞性免疫によって除かれ，第二に，脂肪体で産生され，体液内に分泌される抗菌ペプチドやストレス応答タンパク質などによる体液性免疫によって除去される（Tzou et al., 2002a; Lemaitre and Hoffmann, 2007）．このように抗菌ペプチドは，微生物が体内に侵入してきたときに産生される分泌性のタンパク質であり，ショウジョウバエに限らず，あらゆる生物の免疫機構において非常に重要な役割を果たしている．産生される抗菌ペプチドの種類は，体内に侵入してきた微生物の種類によって異なるが，多様な抗菌ペプチドを産生することによってカビなどの真菌類やバクテリア，ウイルスにいたる幅広い侵入者から生体を防御している．

微生物の感染によって，これら抗菌ペプチドが産生されるメカニズムは，キイロショウジョウバエを用いて詳

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細に調べられている．ショウジョウバエ体内に微生物が侵入すると，バクテリアの細胞壁成分であるペプチドグリカンに結合するペプチドグリカン認識タンパク質（PGRP）などの受容体によって認識され，Toll シグナル経路や Imd シグナル経路などの細胞内シグナル伝達経路を介して脂肪体での抗菌ペプチドの産生を引き起こす（Tzou et al., 2002a; Lemaitre and Hoffmann, 2007）．ショウジョウバエの Toll 経路や Imd 経路は，哺乳類においてもその類似の経路が存在し，関与する因子のいくつかは進化的に高度に保存されている．このように自然免疫に関わる要素は，その機能によって①体内に侵入してきた微生物の認識，②微生物の侵入に応答する遺伝子群の発現を誘導する細胞内のシグナル伝達，③侵入してきた微生物に対して，直接作用することによる体内からの排除（エフェクター）の大きく 3 つのクラスに分類することができる．

昆虫などの無脊椎動物は脊椎動物が持つ獲得免疫を持たないため，自然免疫のみで非常に多様な微生物から自己を防御しなければならない．そのため，エフェクターである抗菌ペプチドは個体の適応度に深く関わり，生物が多様な環境へ適応する過程で非常に重要な役割を果たしてきたと考えられる．そこで，本稿では，ショウジョウバエの免疫機構の中でも，抗菌ペプチドに着目して，その分子進化メカニズムや，抗菌活性と宿主の微生物に対する抵抗性や生態学的な性質との関連性について論じる．

2．ショウジョウバエにおける抗菌ペプチドの特徴

これまでの研究から，数多くの抗菌ペプチドが動物から植物，バクテリアにいたる多様な生物種から同定されており，それら抗菌ペプチドにはいくつかの共通した特徴が見出されている．まず，多くが 100 アミノ酸以下の低分子量で，正電荷を帯びた両親媒性のタンパク質であるため，負電荷を帯びた微生物の細胞膜に直接作用することで排除するモデルが提唱されている（Matsuzaki, 1999; Shai, 1999）．一方，多くの抗菌ペプチドについては，立体構造が明らかになっており，様々な構造が報告されている．例えば，ヒト（*Homo sapiens*）の Cathelicidin (Wang, 2008) やカイコ（*Bombyx mori*）の Moricin (Hemmi et al., 2002) など，α-ヘリックス構造のみからなるものや，ブタ（*Sus scrofa*）の Protegrin 1 (Fahrner et al., 1996) やニホンカブトガニ（*Tachypleus tridentatus*）の Tachyplesin I (Kawano et al., 1990) など β-シート構造のみから成るもの，また，昆虫から哺乳類に至る広範な生物種で存在している Defensin (Bonmatin et al., 1992; Hoover et al., 2001) やヒドラ（*Hydra magnipapillata*）の Hydramacin-1 (Jung et al., 2009) のように，α-ヘリックスと β-シートの両構造を持ち，それぞれがジスルフフィド結合によって折りたたまれた抗菌ペプチドが知られている．その他にも，双翅目昆虫に幅広く存在している Diptericin のようにグリシンやプロリンなど特定のアミノ酸の割合が高いという特徴も報告されており（Wicker et al., 1990），抗菌ペプチド間ではアミノ酸配列レベルでの類似性はほとんど見られない．このような特徴は，抗菌ペプチドの機能や分子進化メカニズムと深く関わっていることが推測される．

昆虫における抗菌ペプチドの機能に関する知見は，Hultmark et al. (1980) によって初めて報告された．彼らは鱗翅目の *Hyalophora cecropia* に対して，*Enterobacter cloacae*（グラム陰性菌）をインジェクションにより感染させると，同じグラム陰性菌である大腸菌（*Escherichia coli*）や緑膿菌（*Pseudomonas aeruginosa*）などに対して *in vitro* で生育阻害を引き起こす低分子のタンパク質の発現が誘導されることを初めて見出した．その後，Steiner et al. (1981) によって，この抗菌活性を示すタンパク質は Cecropin と名付けられた．同様の感染実験によって，Hultmark et al. (1983), Dimarcq et al. (1988) や Lambert et al. (1989) によって Cecropin とは異なる抗菌ペプチドが同定され，それぞれ Attacin, Diptericin, Defensin と名付けられた．これら 4 種類の抗菌ペプチドは，キイロショウジョウバエを用いた感染実験においてもその存在が確かめられ，種を超えて存在することが明らかとなった．これら 4 種類の抗菌ペプチドの抗菌活性は Cecropin の場合と同様に *in vitro* 解析から Attacin と Diptericin はグラム陰性菌に対して，Defensin はグラム陽性菌に対して強い抗菌活性を示すことが確認された（Wicker et al., 1990; Dimarcq et al., 1994; Åsling et al., 1995）．また，ショウジョウバエを用いた詳細な解析により，Cecropin は細菌や真菌類に対して，幅広い抗菌活性を持つことが明らかとなった（Samakovlis et al., 1990; Ekengren and Hultmark, 1999）．一方，キイロショウジョウバエからは，他の昆虫では見つかっていなかった Drosomycin, Metchnikowin, Drosocin の 3 種類の抗菌ペプチドも同定され，*in vitro* の生育阻害実験によって，Drosomycin と Metchnikowin は真菌類に対して，Drosocin はグラム陰性菌に対して強い抗菌活性を示すことが確認された（Bulet et al., 1993; Fehlbaum et al., 1994; Levashina et al., 1995; Charlet et al., 1996）．これらのことから，抗菌ペプチドには種間で共通しているものから，種特異的に存在しているものまで存在すること，また，抗菌ペプチドの種類によって抗菌活性が異なる場合があることが明らかとなった．Tzou et al. (2002b) は，Toll 経路と Imd 経路のノックアウトにより，抗菌ペプチドを産生できなくなったキイロショウ

ジョウバエを作成し，それらにいろいろな抗菌ペプチド遺伝子を導入し強制発現させた．その結果，強制発現させた抗菌ペプチドは，*in vivo* でも *in vitro* で活性を示した微生物種に対して強い抵抗性を示すことが示された．さらに，彼らは，Attacin と Drosocin を共発現させると，大腸菌の感染下における生存率が改善されるなど，発現する抗菌ペプチドの組み合わせによっても微生物への抵抗性は変化することも明らかにし，抗菌ペプチドの発現パターンと微生物の抵抗性の間には深い関係性があることを実験的に証明した．このことから，ある種の微生物の感染から自己を防御するためには，どの抗菌ペプチドを持つかが大きな意味を持つということが分かる．このことから，様々な微生物相を示す環境へのショウジョウバエの適応を理解するためには，ショウジョウバエの進化過程でどのようにして抗菌ペプチド遺伝子のレパートリーが進化してきたかを理解することが大変重要なことであると考えられる．

3．抗菌ペプチドの分子進化

3.1　遺伝子重複による抗菌ペプチド遺伝子の分子進化

2007 年，ショウジョウバエ 12 種の全ゲノム配列が解読され（Drosophila 12 Genomes Consortium, 2007），これら 12 種において免疫に関わる遺伝子がどのように分布し，それぞれがどのような分子進化パターンを示すのかについて網羅的に調べられた（Sackton et al., 2007）．その結果，Toll 経路や Imd 経路を含む細胞内シグナル伝達系に関わる遺伝子はショウジョウバエ 12 種においても高度に保存されており，その多くがゲノム中に単一コピーの遺伝子として存在しているのに対し，PGRP や血球（hemocyte）の細胞膜に存在する nimrod (Kurucz et al., 2007) など，微生物の認識に関わる遺伝子や抗菌ペプチドなどのエフェクターをコードする遺伝子の多くはゲノム中で複数コピー存在し，多重遺伝子族を形成していることが明らかとなった．また，微生物の認識やエフェクターの遺伝子はシグナル伝達系の遺伝子に比べて，系統（種，あるいは種群）特異的に存在するものが多いことも確認された．例えば，キイロショウジョウバエで見つかった 7 種類の抗菌ペプチドについては，Drosomycin はキイロショウジョウバエ種群でのみ，Drosocin は *Sophophora* 亜属でのみ存在が確認されている（図 1）．これらのことから，免疫に関わる遺伝子の中でも，認識やエフェクターの遺伝子はシグナル伝達系の遺伝子とは異なり，進化過程で遺伝子の重複や欠失を繰り返してきたと考えられる．その中でも，抗菌ペプチドは特に多重遺伝子族を形成しているものや，系統特異的なものが多いことが確認されている（Sackton et al., 2007）．

完全変態昆虫に広く分布している Cecropin 遺伝子は全ゲノム配列が決定されたショウジョウバエ 12 種全てで見つかっているが，その数はキイロショウジョウバエで 4 コピー（CecA1，CecA2，CecB，CecC），オナジショウジョウバエ（*D. simulans*）で 3 コピー，*D. grimshawi* で 7 コピー存在し，種間で異なっている．また，これら重複遺伝子はゲノムの狭い領域（10 kb 以内）にタンデムに並んで存在しており，その領域には偽遺伝子化しているコピーも存在している．このことはショウジョウバエの進化過程で活発な遺伝子重複と欠失を繰り返していることを強く示唆している（Ramos-Onsins and Aguade, 1998; Quesada et al., 2005; Sackton et al., 2007）．また，CecA1 遺伝子の上流 600 bp の領域には Cecropin とは異なり，オスの成虫射精管特異

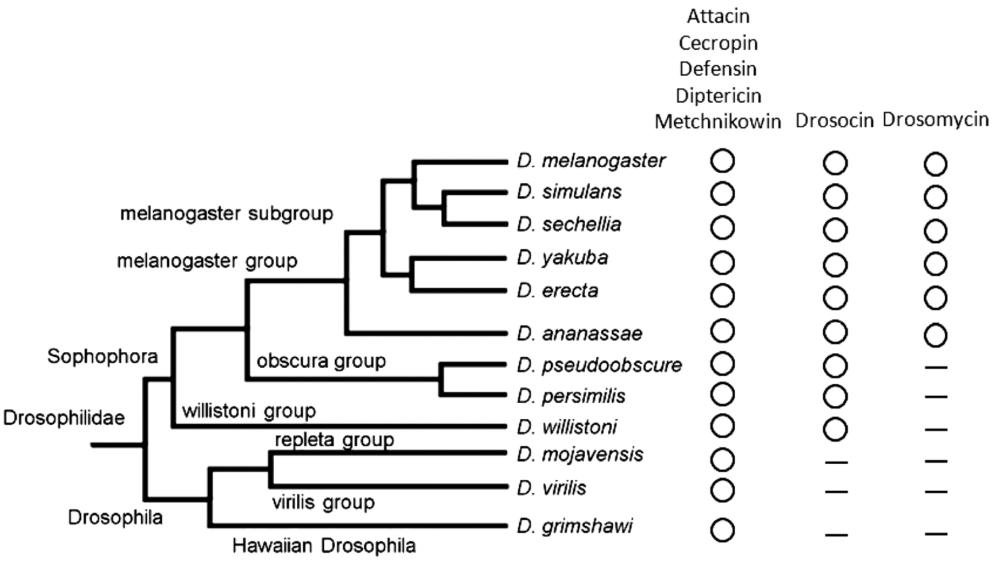

図 1：全ゲノムが解読されたショウジョウバエ 12 種の系統関係と抗菌ペプチドの分布．各ショウジョウバエ種のゲノム中に存在する抗菌ペプチドには丸（○）を，見つかっていないものはマイナス（−）で示してある．

的に発現する抗菌ペプチドであるAndropinの存在が明らかとなっている．Androp inはキイロショウジョウバエ種亜群でのみ見つかっており，2つのエクソンが短いイントロンによって分割される遺伝子構造や，2つのヘリックスを形成するなど，タンパク質の二次構造がCecropinと類似していることから，AndoropinはCecropinの遺伝子重複によって生じ，異なる発現パターンを獲得した新規抗菌ペプチドであることが示唆されている（Date-Ito et al., 2002）．同様にキイロショウジョウバエ種群特異的に存在しているDrosomycinも多重遺伝子族を形成し，キイロショウジョウバエでは7コピー，*D. yakuba*では6コピー，アナナスショウジョウバエ（*D. ananassae*）では4コピー存在し，Cecropin遺伝子と同様に活発な遺伝子重複と欠失によって進化してきたことが示唆されている（Jiggins and Kim, 2005; Sackton et al., 2007）．このように，抗菌ペプチドの進化は，重複と欠失を繰り返す〝birth-and-death evolution〞（Nei et al., 1997）であることが示唆される．しかし，その進化パターンは抗菌ペプチドによって異なる．例えば，Cecropin遺伝子やDrosomycin遺伝子の分子系統解析によると，Cecropin遺伝子は亜群内の近縁な種分化後に高頻度で重複が起こっているのに対し，Drosomycin遺伝子はキイロショウジョウバエ亜群やアナナス亜群の分岐時期に高頻度の重複が起こっていることが明らかとなっている（Jiggins and Kim, 2005; Quesada et al., 2005; Sackton et al., 2007）．これまでに，これら重複抗菌ペプチド遺伝子のコピー間の抗菌活性の違いやコピー数が及ぼす宿主の微生物に対する抵抗性についても調べられてきており，Drosomycinは，コピー数が増加することで真菌類の*Fusarium oxysporum*や*Aspergillus fumigatus*に対する抵抗性が向上することが（Tzou et al., 2002b），また，様々な菌類を用いた*in vitro*での発育阻害実験から，それぞれのコピー間で抗菌活性を示す菌種が異なることが実験的に確認されている（Yang et al., 2006; Tian et al., 2008）．これらのことから，ショウジョウバエの進化過程において，遺伝子重複によって抗菌ペプチド遺伝子のレパートリーやコピー数が変化することが，いろいろな微生物への抵抗性獲得につながってきたと考えることができる．

対照的に，MetchnikowinやDrosocin遺伝子はこれらを持つショウジョウバエのゲノム中，単一コピーで存在しており，CecropinやDrosomycinとは異なる分子進化パターンを示すことも示唆されている（Sackton et al. 2007）．

3.2 抗菌ペプチドにかかる自然選択

免疫に関わる遺伝子は体内に侵入してくる様々な微生物に適応するため，正の選択が働き，進化速度が速くなる傾向があることが分かっている．例えば，脊椎動物において，体内に侵入してきた抗原を認識する主要組織適合遺伝子複合体（major histocompatibility complex, MHC）の抗原認識サイト（antigen recognition site, ARS）は，非同義置換率（dN）が同義置換率（dS）に比べ有意に高い．一方で，MHCのARS以外の領域ではdSの方がdNよりも大きく，同じMHC分子内でも抗原と結合するARSだけが正の選択によって進化していることが明らかとなっている（Hughes and Nei, 1988）．MHCと同様に，哺乳類のDefensinやCathelicidin，ニワトリノのGallinacin，シロアリのTermicinなどの抗菌ペプチドにおいても，dN/dS比の解析から正の選択（dN/dS＞1）が検出されている（Hughes and Yeager, 1997; Bulmer and Crozier, 2004; Lynn et al., 2004; Patil et al., 2004; Tennessen, 2008）．しかし，全ての抗菌ペプチドが正の選択によって進化しているわけではない．ショウジョウバエにおいては，DrosomycinやMetchikowin，Defensinなど，多くの抗菌ペプチド遺伝子では正の選択は検出されていない（Clark and Wang, 1997; Lazzaro and Clark, 2003; Jiggins and Kim, 2005; Obbard et al., 2009）．この要因として，MHCが働く獲得免疫とは異なり，特定の微生物の感染によって様々な抗菌ペプチドが産生されることや，個々の抗菌ペプチドが広範囲の微生物に対する効果を持つことから，抗菌ペプチドは，広い抗菌スペクトルを維持しながら，感染時に効率的に転写・翻訳されることが重要で，そのための純化選択が抗菌ペプチドの進化過程で大きく作用してきた可能性が考えられている（Sackton et al. 2007）．一方，様々な微生物に対する抵抗性の獲得は，進化の過程でそれぞれの種がもつ抗菌ペプチドのレパートリーが変化することによって実現されてきたと思われる．

4. ショウジョウバエの生態学的特徴と抗菌ペプチド

一般的に，キイロショウジョウバエの仲間は果実が発酵した，酵母菌が多い餌を食物としているが，クロショウジョウバエの仲間は川辺や腐敗した樹木などで生育し，バクテリアやカビが多い餌を食物としている．このような生活環境の違いから，クロショウジョウバエはキイロショウジョウバエよりもカビ類に対して強い抵抗性を持つことが必要であると考えられる．実際にアオカビの一種を生やした培地で両方の種の生存率を比較してみたところ，クロショウジョウバエの方が明らかに強い抵抗性を示した（図2）．ところが，これまでにクロショウジョウバエで見つかっている抗菌ペプチド遺伝子の数はキイロショウジョウバエよりも少なく，特に，真菌類に対して強い抗菌活性を示すDrosomycin遺伝子を持っていないことがゲノムデータ解析から示唆されている（表1）．しかし，Sackton and Clark (2009) は，ク

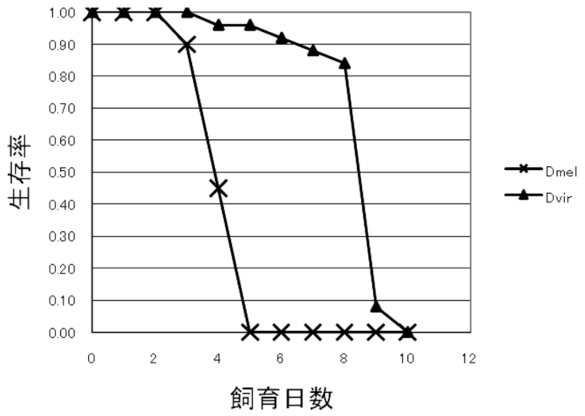

図2：カビの生えた培地で飼育したキイロショウジョウバエとクロショウジョウバエの生存率の差．生存率は各ショウジョウバエの1日目の成虫25個体をアオカビの一種を生やした飼育瓶に入れて測定した．×がキイロショウジョウバエ（Dmel）の生存率を，▲がクロショウジョウバエ（Dvir）の生存率を表している．

表1：キイロショウジョウバエで同定されている抗菌ペプチドの一覧

抗菌ペプチド	主な抗菌活性	遺伝子のコピー数
Attacin	グラム陰性菌	4
Cecropin	グラム陰性菌	4
Defensin	グラム陽性菌	1
Diptericin	グラム陰性菌	2
Drosocin	グラム陰性菌	1
Drosomycin	真菌類	7
Metchnikowin	真菌類	1

ロショウジョウバエにグラム陰性菌の *Serratia marcescens* とグラム陽性菌の *Enterococcos faecalis* を感染させ，それによって発現が誘導された遺伝子を網羅的に解析したところ，クロショウジョウバエにはキイロショウジョウバエでは見つかっていない新規の抗菌ペプチド遺伝子が存在することを示唆した．我々は，アオカビの一種を摂食させることによって発現が誘導される遺伝子をスクリーニングしたところ，同様に新規抗菌ペプチドの可能性がある遺伝子を得た（未発表）．これらの結果から，クロショウジョウバエでは独自の抗菌ペプチドを持つことで，強い抗真菌耐性を獲得した可能性が推察できる．

ショウジョウバエの中には，クロショウジョウバエ以外にも，*Hirtodrosophila* 属や *Mycodrosophila* 属のように菌類のみを食べる菌食性であったり，ハワイ産の *Scaptomyza* 属のショウジョウバエのように菌類や腐敗した樹木，落ち葉や果実などあらゆるものを餌とするなど，非常に多様な食性を示すことが分かっている（Throckmorton, 1975）．また，ショウジョウバエは熱帯から寒帯に至る非常に広い範囲に生息しており，それら生息地における微生物相も多岐にわたると思われる．クロショウジョウバエを用いた実験から示唆されたように，多様な生態的特性を示すショウジョウバエには，未だに同定されていない抗菌ペプチドが数多く存在していることが考えられる．広い範囲のショウジョウバエ種について，それらが持つ抗菌ペプチド遺伝子の特性を調べ，その分子進化がショウジョウバエの生態的特性の進化にどのように関わったのかを明らかにすることにより，ショウジョウバエがどのようにして環境に適応し，進化してきたかを分子レベルで解明することができると期待される．そのためには，クロショウジョウバエとキイロショウジョウバエのように明らかに微生物に対する抵抗性が異なる種を材料に，それぞれが持つ抗菌ペプチドについて分子進化学的解析を行い，また，Tzou et al. (2002b) が行ったように，抗菌ペプチドを産生できないようにしたキイロショウジョウバエ個体に，別の種から得られた抗菌ペプチド遺伝子を導入し，その個体の微生物に対する抵抗性の変化を調べる実験などが有効であると考えられる．

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■紀要「低温科学」の変遷

・低温科學，第 1 輯（1944 年）－第 10 輯（1953 年）
・低温科學．生物篇，第 11 輯（1954 年）－第 35 輯（1978 年）
・低温科学．物理篇，第 11 輯（1953 年）－第 53 輯（1995 年）
・低温科学．物理篇．資料集，第 27 輯（1970 年）－第 63 輯（2005 年）
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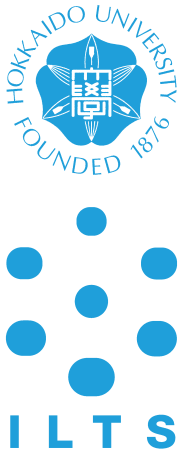

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