
Reproductive mode and split sex ratios in the facultatively ovoviviparous thrips, *Bactrothrips brevitubus*

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ABSTRACT

Researching the evolution and maintenance of reproductive mode (oviparity, ovoviviparity and viviparity) is important for identifying the intrinsic and environmental pressures associated with maternal care. We studied the life history, reproductive mode and sex allocation of *Bactrothrips brevitubus*, a haplodiploid, facultatively ovoviviparous Japanese thrips. Individual females can produce a mass of eggs oviparously and single eggs ovoviviparously. In our study, about 30% of the females reproduced exclusively by oviparity. Nearly 60% of the oviparous females also reproduced ovoviviparously. Local resource competition between reproductive females for food and egg-laying sites may select for ovoviviparity, as females can retain the eggs until a better rearing site is located. We also have preliminary evidence that predatory mites promote ovoviviparity, as they consume the eggs but are ectoparasitic on larvae and adults. Another factor that may influence reproductive mode is that females can produce both sexes oviparously but only males ovoviviparously. Consequently, sex allocation and reproductive mode strategies act in tandem. The population-wide sex ratio was unbiased, whereas the sex ratio of broods that were produced at least partly oviparously was female biased. This female-biased sex ratio was not significantly different from that predicted by Godfray's split sex ratio model for a panmictic population. Even so, our evidence for local mate competition and local resource competition suggests that the sex ratios were not Fisherian. We predict that females should reproduce oviparously when constrained by local mate competition, ovoviviparously when constrained by mite predation, with or without local resource competition, and use a mixed strategy when constrained by mite predation and local mate competition.

Keywords: egg mass, haplodiploidy, ovoviviparity, reproductive mode, split sex ratios, thrips.

INTRODUCTION

Internal fertilization and egg retention are major 'advances' in the evolution of maternal care (Alcock, 1998). These adaptations have increased a female's control over her fitness but have reduced paternal certainty and the reliability of paternal care (Clutton-Brock, 1991;

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Tallamy, 2000). Females who retain their eggs, as in ovoviviparous (partial embryonic development within the mother) and viviparous (live bearing) species, have greater control in protecting their offspring during early development. Such species occur among groups of reptiles (Shine, 1985), fishes (Breder and Rosen, 1966; Wourms, 1977), amphibians (Wells, 1981), gastropods (Baur, 1994) and many arthropods (Hagan, 1951). Facultative ovoviviparity, as in idolothripine thrips, enables an individual female to choose her reproductive mode according to the risks and trade-offs of egg laying (oviparity) and retention (Hagan, 1951; Crespi, 1993). Studies on such species are useful for investigating the selective pressures influencing reproductive mode and parental care.

Explanations for the evolution and maintenance of ovoviviparity and viviparity include: (i) extreme climates and environments, as in reptiles (Shine and Bull, 1989) and gastropods (see references in Baur, 1994; Hughes, 1995); (ii) limited resources, as in fishes (Macfarlane *et al.*, 1993), salamanders (Dopazo and Korenblum, 2000) and thrips (Ananthakrishnan *et al.*, 1983a,b; Dhileepan and Ananthakrishnan, 1987); (iii) protection from desiccation, as in gastropods (see references in Baur, 1994; Heller *et al.*, 1997) and thrips (Ananthakrishnan *et al.*, 1983b); (iv) predation, as in gastropods (see references in Baur, 1994) and insects (Vishniakova, 1968, cited in Roth, 1989; Kudo *et al.*, 1995; Meier *et al.*, 1999); and (v) ephemeral or unpredictable habits, as in reptiles (Tinkle and Gibbons, 1977, cited in Shine, 1985) and dipterans (see references in Meier *et al.*, 1999). More specifically, in the facultatively viviparous thrips, *Elaphrothrips tuberculatus*, where females are produced oviparously and males are produced viviparously, larger females produce mostly by viviparity. This is apparently because large females produce large males who have greater fitness returns from being large than do females (Crespi, 1988, 1989).

In all thrips species, females are diploid and males are haploid (Stannard, 1968; Lewis, 1973; Crespi, 1991). Females develop from fertilized eggs and males develop from unfertilized eggs. Embryogenesis in 'typical' ovoviviparous thrips occurs in the lateral oviducts up to blastokinesis (Haga, 1975; Dhileepan and Ananthakrishnan, 1986). Such oocytes are unlikely to be fertilized by sperm entering the common oviduct (distal to the lateral ducts) from the spermatheca. Therefore, ovoviviparous oocytes are likely to become male offspring. This constraint has important consequences for resource and sex allocation at the individual and population-wide levels, as there may be trade-offs between the best reproductive strategy and the best sex-allocation strategy. Such potential trade-offs mean that the qualities of haplodiploidy and facultative ovoviviparity make thrips excellent study organisms for investigating the relationship between reproductive mode and sex allocation.

More than 20 thrips species in the subfamily Idolothripinae have been identified as facultatively ovoviviparous or viviparous (for example, see references in Hagan, 1951; Ananthakrishnan *et al.*, 1984; Crespi, 1993; Kucharczyk, 1993). However, there have been few studies on the life history of these species (e.g. Haga, 1974, 1980; Ananthakrishnan *et al.*, 1983a,b; Dhileepan and Ananthakrishnan, 1987) and even fewer (Ananthakrishnan *et al.*, 1984; Crespi, 1988, 1989, 1993) have investigated reproductive mode with respect to sex allocation.

Of the more than 40 species of idolothripine *Bactrothrips* Karny in the world (Mound and Palmer, 1983), seven have been described in Japan (Haga and Okajima, 1989). Females facultatively lay egg masses oviparously and single eggs ovoviviparously (Haga, 1974). This paper explores the life history of one of these species, *B. brevitybus* Takahashi. It examines when and why ovoviviparity occurs. We consider how reproductive mode and sex allocation

interact at the individual and population-wide levels and what the thresholds might be for a female in 'assessing' her best reproductive strategy. To achieve this, we investigated reproductive mode with respect to sex ratio, food and space availability, body size, predation pressure and apparent population structure. Based on our data and observations, we make specific predictions about the preferred reproductive strategy under different reproductive and sex allocation constraints.

MATERIALS AND METHODS

Study organism

Like all *Bactrothrips*, *B. brevitubus* is sporophagous. It feeds mainly on *Pestalotia parmarum* spores (Haga, 1972) that grow on the dead hanging leaves of evergreen *Castanopsis*, *Quercus*, *Cinnamomum*, *Machilus* and *Neolitsea* trees (Haga, 1974; Haga and Okajima, 1989). Haga (1980) observed that males guard their egg mass. He also observed that major predators of these eggs are mites, but he did not observe males chasing mites from an egg mass.

Study sites and field collections

Individuals and colonies, or aggregations, of *B. brevitubus* were collected approximately monthly from September 2000 to August 2001 at each of two sites in the greater Tokyo area: Jinmuji Forest, approximately 40 km south of Yokohama, and Mt. Koma, approximately 45 km west-southwest of Jinmuji. The host trees at Jinmuji and Koma are *Quercus glauca* and *Cinnamomum camphora*. A total of 107 colonies (used here also to refer to aggregations), consisting of adults and/or juveniles, were collected from Jinmuji and 174 colonies were collected from Koma.

On each field trip, dead hanging host leaves were examined individually for adults, eggs and juveniles. Approximately 70% of the colonies found were collected. Where a large stand of dead hanging leaves was located (> 150 leaves), the stand was initially bashed with a stick over a collecting cloth to determine whether thrips were present. If thrips were found, individual leaves were checked and some of the colonies were collected. All thrips on a leaf, or two adjacent leaves, were put into a zip-lock bag that contained a moist paper ball to maintain humidity. Thrips on dead leaves that were more than about 3 m from the ground were not collected, even though thrips were found at this height. This further ensured that thrips were left in the area. Samples were transported to the Tokyo University of Agriculture (TUA), about 2 h travel from each site, at ambient temperature.

Basic life history

At TUA, the thrips were examined immediately under a dissecting microscope (Olympus SZX9, Tokyo, Japan). Adults were sexed. Males were easily distinguished from females by their prominent tubercles on abdominal segment VI (Haga and Okajima, 1989). The number of eggs in a mass, or alone, and the numbers of each developmental stage were recorded. The number of predatory mites was also recorded. Colonies were individually placed in 8 × 5 × 1 cm rearing dishes. The dishes had a substrate of 10% granulated charcoal

in a plaster base to maintain moisture. About half of these colonies were reared at room temperature to observe development and behaviour and about one-quarter were dissected (the other colonies were frozen for future DNA analyses).

Ovarian dissections were performed on a subsample of females ($n = 139$) within 2 days of collection. Using an Olympus BH2 compound light microscope (Tokyo, Japan), the length and width of the three largest oocytes was measured to $\pm 2.5 \mu\text{m}$. The number of chorionated oocytes was recorded. When oocytes were developed, they were scored as oviparous or ovoviviparous. In some of these females, the length and width of the spermatheca was measured ($n = 94$). Insemination status was determined by puncturing the spermatheca and observing it with a light microscope or by observing the intact spermatheca under Nomarski illumination ($n = 129$). The approximate density of sperm in the spermatheca was categorized from zero (uninseminated) to 5 ('full' of sperm) ($n = 71$). Using the same scale, the approximate density of fungal spores in the gut of some gravid females was categorized ($n = 17$ oviparous and 13 ovoviviparous females).

The sex of well-developed ovoviviparous embryos, which were effectively young first-instar larvae, was determined by examining their abdominal setae (chaetotaxy). First-instar larvae of all female thrips have three pairs of setae on the dorsal aspect of their ninth abdominal segment, whereas males have four pairs (Heming, 1991; Kranz *et al.*, 1999). Well-developed embryos were carefully dissected from their chorion, mounted dorsally on a microscope slide in Burlese mounting medium and cleared at 50°C for 24 h ($n = 37$ embryos from 14 females). The chaetotaxy of the ninth abdominal segment was examined at $200\times$ magnification.

The body size of most females that we dissected was determined by measuring head length and head width, just posterior to the base of the eyes ($n = 71$). The head was measured because, unlike body length, it is not prone to changes in size with diet and reproductive status.

Sex ratio

The adult sex ratio was determined from field collections. The sex ratio was also determined for the offspring of these adults, which were reared in the laboratory after collection. Most of these offspring were sexed after adult eclosion. If an individual died as a first-instar larva or pupa, they were sexed using chaetotaxy (Heming, 1991). Dead second-instar larvae, which accounted for about 20% of all juvenile mortality, were not sexed because the chaetotaxy in this stage is sexually monomorphic (Heming, 1991).

Data analyses

All data were analysed using SPSS[®] version 10.0.5. Homoscedastic data, assessed using Levene's test, were analysed with t -tests or an analysis of variance (ANOVA). As leaf sex ratios were highly truncated, even after arcsine transformation, non-parametric tests were used to analyse these data. For branch sex ratios, the data were arcsine transformed before being subjected to t -tests. Other heteroscedastic or categorical data were assessed using non-parametric tests. In this study, $\alpha = 0.1$, but data at $0.05 < P < 0.1$ are described as 'marginally significant'. The results are reported throughout the text as the mean \pm standard error.

RESULTS

Basic life history

The general life cycle of *B. brevitubus* was similar at Jinmuji Forest and Mt. Koma (Fig. 1). Adults of both sexes overwintered from approximately December until late February. We frequently observed overwintering adults aggregating on a single leaf or on adjacent leaves, and in several cases the remnants of an old conspecific egg mass was on the same leaf. After overwintering, adults reproduced from spring (March) through summer and into autumn (Figs 1, 2). The average time that an individual took to develop from an egg, collected in the field, to adult eclosion, in the laboratory, was 31 ± 0.8 days ($n = 28$). These data, combined with three distinct peaks of egg production at both sites (Fig. 1), indicate that *B. brevitubus* is multivoltine, with about three generations per year.

Egg masses were observed at both sites during the reproductive season. The average number of eggs in a mass before any had hatched was 22 ± 2 (range = 6–64). We observed both single males and single females guarding egg masses, but neither sex guarded larvae. Occasionally, a male was present with a female while she guarded, but the male always appeared to be guarding the female rather than the egg mass. Adults were observed to chase away psocopterans but not mites. Single ovoviparous eggs were rarely observed in the field. This is not surprising because single eggs in the laboratory hatched within a day or two of being laid. Adults were usually observed alone or with one or two adults of the opposite sex (see below), but in some cases were found in groups of four or more. However, there was no significant difference in the number of adult females (one to four) versus the total number of juveniles on a leaf (or adjacent leaves) at either Jinmuji (Kruskal-Wallis $\chi^2_3 = 0.435$, $P = 0.933$, $n = 48$) or Koma ($\chi^2_3 = 2.299$, $P = 0.513$, $n = 95$). There was also no difference in the number of females versus the number of eggs in a mass (Jinmuji: $\chi^2_3 = 3.541$, $P = 0.315$, $n = 35$; Koma: $\chi^2_3 = 1.406$, $P = 0.704$, $n = 98$).

Reproductive mode

Ovarian dissections of females indicated that oviparity and ovoviviparity were common reproductive modes at both sites from mid- to late spring through early autumn (Fig. 2). There was no significant difference in the mean of the three longest oocytes of oviparous females between the sites from spring to autumn ($311 \pm 34 \mu\text{m}$ at Jinmuji and $344 \pm 80 \mu\text{m}$ at Koma; t -test, $t_{46} = 0.391$, $P = 0.697$). All females collected at Jinmuji from October to March had undeveloped oocytes (mean of the three longest oocytes per female = $131 \pm 9 \mu\text{m}$; $n = 16$ females). All but five females collected at Koma from November to April had undeveloped oocytes ($150 \pm 6 \mu\text{m}$; $n = 44$). The five females with some development had chorionated, apparently oviparous oocytes, but the oocytes were too small ($291 \pm 18 \mu\text{m}$) to be certain of their eventual mode of reproduction. Of the dissected females with developed oocytes, 21 (28%) of the 75 dissected had between one and 16 well-developed ovoviviparous oocytes; all others had chorionated oviparous oocytes.

Individual females produced both oviparous and ovoviviparous offspring. Thirty-two (58%) of the 55 females collected from the field with an egg mass produced one or more single ovoviviparous eggs when subsequently reared in the laboratory. It is possible that some of these females did not lay the egg mass that they were collected with. However, there was only ever one female on a leaf with an egg mass and in each case the female guarded the mass, suggesting that she was the mother.

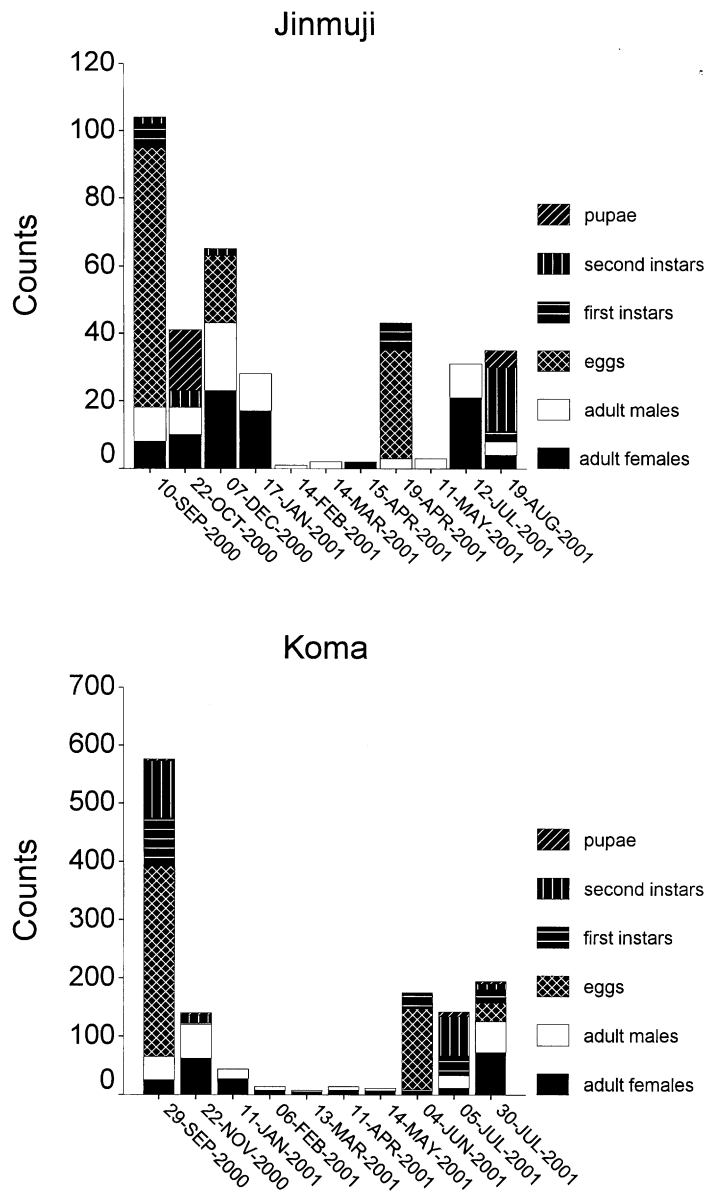


Fig. 1. Basic life cycle of *Bactrothrips brevitubus* at Jinmuji Forest and Mt. Koma between September 2000 and August 2001. Data show total counts of developmental stages and sexes of adults. At both sites, reproduction occurred from spring through autumn, with the highest population numbers in late summer. Adults overwinter.

Nearly all females collected from the field had only oviparous or ovoviviparous oocytes at the time of dissection. However, one female collected on 15 April from Jinmuji and two females collected on 15 May from Koma had both types of oocytes. These females had

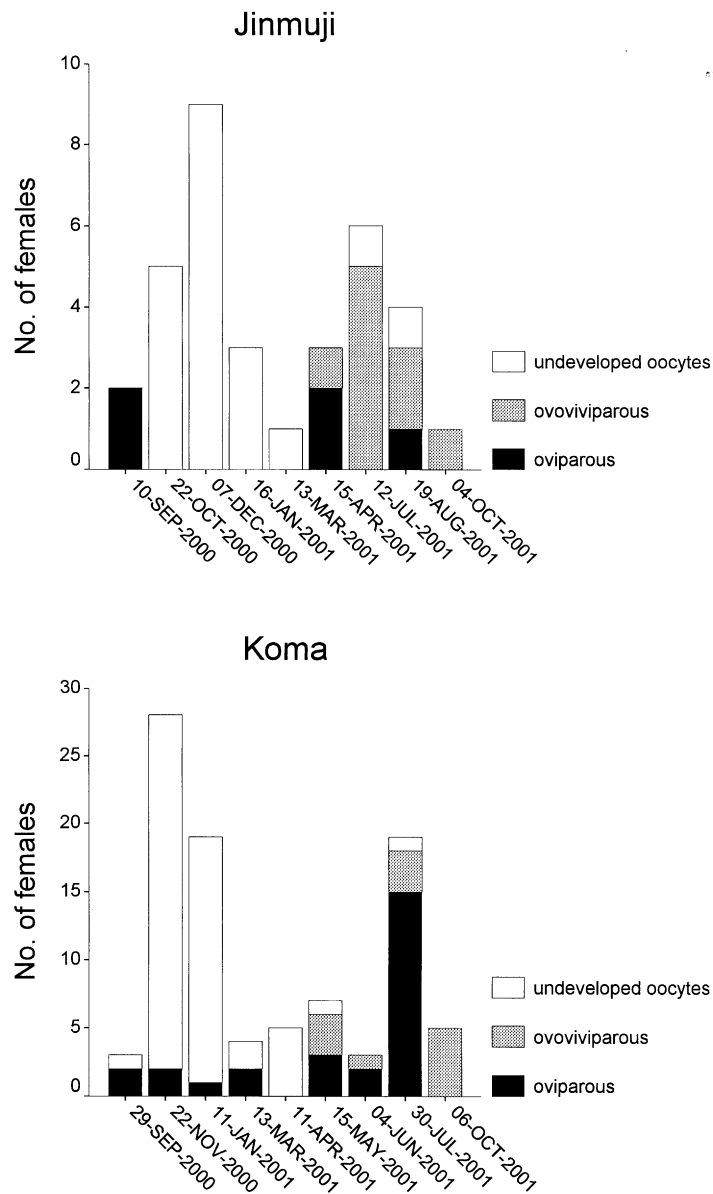


Fig. 2. Reproductive mode of *Bactrothrips brevitubus* at Jinmuji Forest and Mt. Koma between September 2000 and August 2001, determined by ovarian dissections. Undeveloped oocytes were those of females where the mean of the three longest oocytes was less than 200 μm . Ovoviviparous oocytes were those of gravid females where embryos were developing. Oviparous oocytes were those of gravid females with no embryonic development. The five females designated as oviparous at Mt. Koma between November and April had partly developed oocytes ($291 \pm 18 \mu\text{m}$; mean \pm standard error). Although they appeared to be oviparous, the oocytes were too small to be certain of their eventual reproductive mode.

distinct embryos in one or more of their oocytes and also had well-developed oviparous oocytes. They differed from other ovoviviparous females in that their most developed oocytes (i.e. those in the distal portion of the lateral ducts) were oviparous, indicating that these females had changed reproductive mode. Conversely, the most distal oocytes of all other ovoviviparous females had nearly fully developed first-instar juveniles. Indeed, four females, two collected on 4 June from Koma and two collected on 19 August from Jinmuji, had a fully developed first-instar larva in their common oviduct (i.e. they were viviparous). The chorionic sac was absent around each juvenile and they were not distinguishable from newly hatched first-instar larvae.

Our results indicate that only males are produced ovoviviparously. All sexed ovoviviparous embryos were male ($n = 37$ embryos from 14 females). Moreover, all of the offspring produced from single eggs in the laboratory were male ($n = 56$ males from 21 females).

Mating patterns and insemination status

In the laboratory, we observed both sib-mating and apparent random mating between newly eclosed individuals and those that had had previous mates. This was regardless of whether the individuals were familiar with each other before the sexual encounter. Of the females dissected after collection from the field, 119 (92%) of 129 were inseminated. All overwintering females were inseminated. Only one of the 47 gravid females was not inseminated, and she was ovoviviparous. There was no significant difference in the volume of spermatheca between females with developed oviparous oocytes ($0.031 \pm 0.004 \text{ mm}^3$, $n = 15$) and those with developed ovoviviparous oocytes ($0.029 \pm 0.009 \text{ mm}^3$, $n = 7$) (t -test, $t_{20} = 0.209$, $P = 0.836$). There was also no difference in the sperm-density category of these females ($t_{34} = 0.777$, $P = 0.442$).

Body size

A two-way ANOVA was performed to determine whether there was a relationship between the size of a female and her reproductive mode. Female head surface area (calculated simply as width \times length) was a fixed factor, collection date was a covariate, and the dependent variable was reproductive mode, which included all gravid females with oviparous or ovoviviparous oocytes. There was no interaction between head size and collection date (ANOVA, $F_{1,47} = 0.500$, $P = 0.853$). An independent t -test on these data revealed no significant difference in size between the two reproductive modes (mean head size: oviparous females = $0.159 \pm 0.004 \text{ mm}^2$, $n = 32$; ovoviviparous females = $0.162 \pm 0.005 \text{ mm}^2$, $n = 18$; $t_{48} = -0.501$, $P = 0.618$). However, there was a marginally significant positive correlation between female head size and brood size (excluding females that were with one or more other females on a leaf) (Pearson $r = 0.213$, $P = 0.079$, $n = 69$). In ovoviviparous females, there was also a significant positive correlation between head size and the mean volume of the three largest oocytes (calculated as a closed cylinder from the length and diameter of the oocytes) (Pearson $r = 0.496$, $P = 0.036$, $n = 18$ females). The latter test was not performed on oviparous oocytes because they were not retained in females long enough for the data to be meaningful.

Spore density, mite predation and field aggregations

The effect of spore density and predatory mites on reproductive mode was examined. As the spore density category data were heteroscedastic, and some of the density categories had < 5 data, non-parametric rank tests were performed. There was a marginally significant difference between spore gut densities for collection date (Kruskal-Wallis $\chi^2 = 8.824$, $P = 0.066$). The gut spore density rank was significantly higher in oviparous females than in ovoviviparous females (Mann-Whitney $U = 56.5$, $P = 0.013$; Fig. 3).

Predatory mites were observed to consume eggs of *B. brevitybus*, but were ectoparasitic and non-fatal to larvae, pupae and adults. The mites were not identified, but probably belonged to the Acarophenacidae. Mites in this family are known predators of idolothripine eggs, and are ectoparasites of these juveniles and adults (A. Goldarazena, personal communication). There was no significant difference in the total number of mites on broods that had been produced oviparously and ovoviviparously ($t_{49} = 0.771$, $P = 0.481$), or when the number of mites was divided by brood size (i.e. mites per individual) ($t_{49} = 0.602$, $P = 0.550$). However, the number of mites (> zero) was positively correlated with the proportion of males in the F_1 generation (offspring of adults collected in the field) (Spearman rank $r = 0.667$, $P = 0.007$; $n = 15$; Fig. 4). A partial correlation of these factors, controlling for brood size, was also significant ($r = 0.719$, $P = 0.006$).

Throughout our field collections, we noted that when there were many (e.g. more than three) females on a small branch, there appeared to be fewer egg masses per capita than when females were found alone or with a few other females. We did not count all of the adults at each site, but we divided the females that we dissected into two groups: those in a group of fewer than four females and those in a group of four or more females. A 2×2 contingency chi-square analysis revealed that there were more ovoviviparous females than

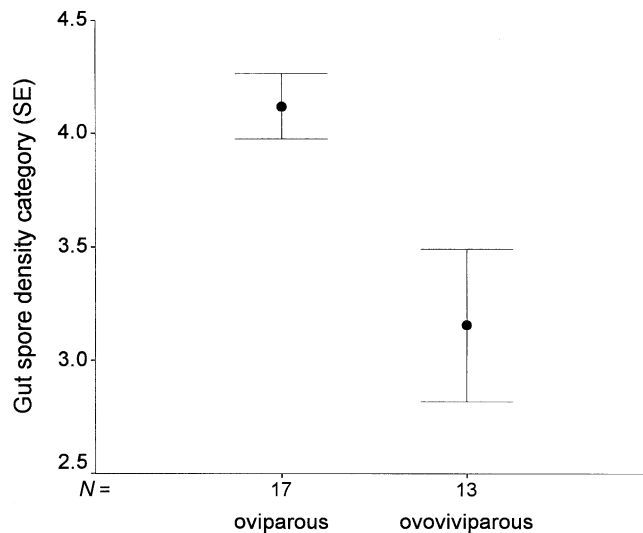


Fig. 3. Mean gut spore density, categorized from 1 to 5, with increasing density, of gravid females with oviparous and ovoviviparous oocytes. Oviparous females had a significantly higher spore density category than ovoviviparous females ($P = 0.013$). SE = standard error.

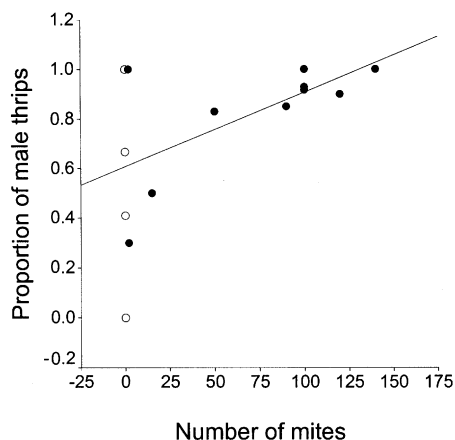


Fig. 4. Correlation between the number of mites on a thrips brood and the offspring sex ratio of adults collected in the field ($r = 0.667$, $P = 0.007$, excluding data for zero mites). The correlation remains significant when the correlation controls for brood size ($P = 0.006$). $n = 15$ colonies with mites. ●, mites; ○, no mites.

predicted at sites with four or more females and more oviparous females at sites with fewer than four females ($\chi^2_1 = 5.526$, $P = 0.019$; $n = 33$ oviparous females from 25 groups and 20 ovoviviparous females from 12 groups).

Sex-specific distribution and sex ratios

Sex ratio data were calculated and analysed in three ways. First, the sex-specific distribution of adults on a single leaf, or two adjacent leaves, was determined. All further reference to a single leaf also includes adults on adjacent leaves; there were never thrips on more than two adjacent leaves. The data were highly truncated at 0 (all females) and 1 (all males), even after arcsine transformation. Hence, all analyses of these data were performed with non-parametric tests. Second, the sex ratio was determined for all adults on a branch or tree. Third, the sex ratio of the F_1 generation (the offspring of adults collected in the field and subsequently reared in the laboratory) was determined. We present each of these sex ratios below.

The mean adult sex ratio (proportion of males) on a leaf was 0.48 ± 0.05 ($n = 116$ females and 115 males) at Jinmuji and 0.47 ± 0.03 ($n = 230$ females and 221 males) at Koma. To compare the distribution of adult *B. brevotubus* on leaves at Jinmuji and Koma, census data were categorized as: (i) only juveniles; (ii) single females; (iii) single males; (iv) single females with single males; (v) multiple females, no males; (vi) multiple males, no females; (vii) multiple adults, female bias; (viii) multiple adults, male bias. As there were only three leaves with multiple adults and no sex ratio bias (all with two males and two females at Koma), these leaves were not included in the analysis. Based on these categories, there was no significant difference in the distribution of the thrips between the two sites ($\chi^2_7 = 8.37$, $P = 0.302$), and so these data are further considered together. Juveniles were present without any adults on 40 (14%) of the 281 leaves. Sixty per cent of the leaves with adults had a single adult; 75 leaves (27%) had a female and 69 (24%) had a male. Twenty-seven leaves (11% of the leaves

with adults) had one male and one female together. Five leaves had more than 10 adults together and all of these had a female bias. Even though the distribution of sexes varied among leaves, there was no significant difference between the sex ratio of adults on a leaf with an egg mass compared to those without an egg mass (Mann-Whitney $U = 2470$, $P = 0.735$; $n = 30$ leaves with an egg mass, 171 leaves without an egg mass).

The second way that sex ratios were determined was by grouping all adults collected from a branch. The overall mean sex ratio (r) of these data was not significantly different to parity (0.5) at either Jinmuji ($r = 0.51 \pm 0.07$; one-sample t -test on arcsine transformed data against 0.5, $t_{23} = 1.242$, $P = 0.228$) or Koma ($r = 0.46 \pm 0.05$; $t_{26} = 0.229$, $P = 0.767$). There were no differences in sex ratio between collection times at Jinmuji (Kruskal-Wallis $\chi_{13} = 18.7$, $P = 0.131$) or Koma ($\chi_{10} = 15.6$, $P = 0.111$), although the small sample sizes for each collection time ($n = 2-4$) mean that the power of these tests was low. As for leaf sex ratios, there was no significant difference in branch sex ratios between Jinmuji and Koma (Mann-Whitney $U = 325.5$, $P = 0.661$).

The overall sex ratio of the F_1 generation reared in the laboratory (the offspring of adults collected in the field, which comprised individuals collected as eggs or juveniles in the field and those from eggs laid in the laboratory) was $r = 0.55 \pm 0.05$ male ($n = 41$ females and 43 males from 72 colonies). This sex ratio is not significantly different from parity (Mann-Whitney U corrected for ties against 0.5 = 2232; $P = 0.114$). The distribution of the sex ratios was similar to those of adults on leaves in the field, in that it was heavily truncated at $r = 0$ and $r = 1$ ($n = 22$ females for $r = 0$ and 28 males for $r = 1$; Fig. 5). There was a marginally significant difference between the means of these laboratory and field sex ratios (Mann-Whitney $U = 7722$, $P = 0.076$). Of the 28 colonies with $r = 1$, 21 (29% of the 72 colonies) were produced from only single, ovoviviparous eggs, which is similar to the 28% of

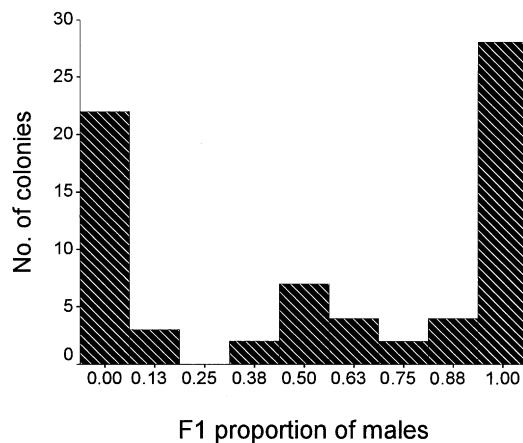


Fig. 5. Frequency distribution of the offspring of adults collected in the field ($n = 72$ colonies) and subsequently reared in the laboratory. Twenty-one of the 28 colonies with $r = 1$ (29% of all the colonies) were produced only from ovoviviparous eggs. This is similar to the 28% of females with ovoviviparous oocytes in the field. The overall mean sex ratio (0.55 ± 0.05) was not significantly different from parity. The mean sex ratio of colonies that were produced at least partly oviparously was 0.37 ± 0.05 male. This sex ratio is not significantly different from that predicted by Godfray's (1990) formula for calculating the sex ratio of unconstrained females in a panmictic population ($P = 0.854$).

females with ovoviviparous oocytes in the field. Of these 21 colonies, only one of the mothers was uninseminated. Of the seven colonies with $r = 1$ (all male) that were produced at least partly from an egg mass, three mothers were uninseminated. Due to haplodiploidy, their broods were all male. The other four colonies had high mortalities (12–80%); as not all of these juveniles were sexed, it is possible that some of them were female.

The mean sex ratio of laboratory-reared colonies that were produced at least partly oviparously was 0.37 ± 0.05 male. We compared this sex ratio to Godfray's (1990) formula for calculating the sex ratio of unconstrained females (i.e. capable of producing offspring of both sexes) under panmictic conditions. Godfray's formula is: $0.5(1 - 2p)/(1 - p)$, where p is the proportion of constrained (in this case, exclusively ovoviviparous) reproductive females in the population. For the 0.28 (laboratory) and 0.29 (field) proportion of exclusively ovoviviparous females, described above, the predicted sex ratio for unconstrained females is 0.28 and 0.27 male, respectively. The mean sex ratio of the laboratory-reared offspring was not significantly different to 0.27 (Mann-Whitney U corrected for ties against 0.27 = 1275; $P = 0.854$) and was significantly female biased (Mann-Whitney U corrected for ties against 0.5 < 0.001; $P < 0.001$).

DISCUSSION

Females of *Bactrothrips brevitubus* can produce both sexes oviparously but are constrained to produce only males ovoviviparously. Our data indicate that the all-male broods of ovoviviparous females were not a result of sperm limitation, as insemination status was not associated with reproductive mode. The constraint is most likely due to the spermathecal duct being in the common oviduct, where only fully, or almost fully, developed embryos enter before an oocyte is laid. Of the few other thrips where reproductive mode and sex allocation have been studied, *Sporothrips amplus*, like *B. brevitubus*, produces both sexes oviparously and only males viviparously (Crespi, 1993), whereas *Elaphrothrips tuberculatus* (Crespi, 1988, 1989), *E. coniferatum* and *Idolothrips spectrum* (Crespi, 1993) produce only males viviparously and only females oviparously. *Dinothrips sumatrensis* is apparently entirely viviparous, and is unusual because all broods are female (Ananthakrishnan *et al.*, 1984).

The association between reproductive mode and sex allocation resulted in a split sex ratio. The constrained (exclusively ovoviviparous) females produced only males and the unconstrained (at least partly oviparous) females produced a female-biased sex ratio. The combination resulted in a population-wide sex ratio that was unbiased.

Selection for reproductive mode

Our data do not indicate a relationship between body size and reproductive mode in *B. brevitubus* during any season. This contrasts with *Elaphrothrips tuberculatus*, in which viviparous females (that produce only males) are larger than oviparous females (that produce only females) in early summer (Crespi, 1988, 1989). It also contrasts with four other species of facultatively ovoviviparous and viviparous idolothropines (Dhileepan and Ananthakrishnan, 1987); the sex allocation of these species was not specified. Crespi's (1988, 1989) data indicated that *E. tuberculatus* males gain greater direct fitness advantages from being large than females. As he also found that large females produce large offspring, large females should have greater fitness from producing males. For *E. tuberculatus*,

therefore, large females will have greater second-generation fitness by being ovoviviparous. However, *B. brevitubus* females can produce males using both reproductive modes. Therefore, any selection for large females to produce males need not be linked to ovoviviparity. In any event, both fecundity and offspring size were positively correlated with body size in females of *B. brevitubus*, and this should be investigated further, together with male body size and fitness.

Crespi (1988, 1989) and Dhileepan and Ananthkrishnan (1987) found that oviparous females had higher fecundity than viviparous females in the idolothripines that they studied. This trend also occurs in beetles (Kudo *et al.*, 1995), amphibians (Wake, 1993) and gastropods (Baur, 1994). We could not examine the fecundity consequences of reproductive modes in *B. brevitubus* because of high mortality among first-instar larvae. This needs to be done to understand the trade-offs associated with each reproductive mode.

In *E. tuberculatus*, viviparous (large) females occur in patches with higher fungal fruiting body density than oviparous females (Crespi, 1988), perhaps because larger females require more food. Conversely, in *B. brevitubus* we found that oviparous females had a significantly higher density of spores in their gut than ovoviviparous females. Also, our field observations and dissections indicate that females in aggregations are more likely to be ovoviviparous than those in small groups or in isolation. This phenomenon has been observed in another idolothripine, *Tiarothrips subramanii* (Ananthkrishnan *et al.*, 1983b). Taken together, our data suggest that competition among females for resources (food and possibly space) could promote ovoviviparous reproduction. Presumably, a female with higher food intake is either in a more reliable habitat or is competitively superior. In either case, it could be a good strategy to produce as many offspring as possible, which is cheaper and faster to do oviparously. It also appears to be necessary for an oviparous female to have her 'own leaf', where she can produce an egg mass. Consequently, competition for suitable leaves (e.g. adequate spore density) might promote ovoviviparity.

Another, non-exclusive explanation for the relationship between aggregation and reproductive mode could be that aggregation promotes high densities of predatory mites. Our laboratory data are somewhat ambiguous regarding reproductive mode and the role of predatory mites. There was no significant difference between the number of laboratory-reared mites on broods from egg masses (oviparity) and those from single eggs (ovoviviparity). However, the number of mites was positively correlated with the proportion of males in the F₁ (laboratory-reared) generation, even when the effect of brood size was accounted for. We cannot exclude the possibility of sex-specific mortality associated with mite predation. However, the relationship between predation, resource availability, sex ratio and reproductive mode warrants further investigation in this species for at least four reasons. First, these mites kill and consume the eggs of all Japanese *Bactrothrips* eggs, but are ectoparasitic and non-fatal to the larvae, pupae and adults of these thrips (B. Kranz and T. Shibata, unpublished observations). Consequently, a female might reproduce ovoviviparously when predatory mites are a threat, and oviparously when predatory mite pressure is low. Second, when a female reproduces both oviparously and ovoviviparously, as did 58% of the females that produced an egg mass in this study, a statistical difference in the number of mites for each reproductive mode might not be detectable at a given time. A mixed strategy (see below and Table 1) could partly account for the lack of difference in the number of mites on broods produced by the two reproductive modes in this study. Third, predation has been suggested as a factor in the evolution or maintenance of ovoviviparity in snails (Heller *et al.*, 1997), cockroaches (Vishniakova, 1968, cited in Roth, 1989), beetles (Kudo *et al.*, 1995) and

Table 1. Predictions of reproductive mode for females without and with reproductive and sex allocation constraints

Constraint	Oviparity	Ovoviviparity	Mixed strategy
None	x		
LMC	x		
LRC		x	
Predatory mites		x	
Mites, LRC		x	
Mites, LMC			x
Mites, LRC, LMC			x

Note: These predictions assume that, other things being equal, oviparity requires less maternal investment than ovoviviparity, because males are involved in egg guarding, and that overall fecundity is higher. Local mate competition (LMC) selects for a female bias, and only oviparous females can produce females. Local resource competition (LRC) selects for a male bias; here ovoviviparity is predicted because females can continue to search for a better feeding site while their embryos are developing, and they do not exclusively require a good-quality leaf for laying an egg mass. Ovoviviparity is predicted with predatory mites because only eggs are consumed. When mite pressure and local mate competition occur together, with or without local resource competition, a mixed strategy is predicted.

gastropods (Baur, 1994), and viviparity in reptiles (Neill, 1964; Shine and Bull, 1989). Indeed, Shine and Bull (1989) suggested that egg retention is more likely to evolve in reptiles where females guard their eggs, which is the case for *B. brevitubus*, as the cost–benefit of investment in their embryos is already high. Fourth, several predation and foraging models (e.g. Abrams, 1993, 1994; Brown, 1999) indicate that there is likely to be a range of trade-offs for individuals when they assess the risk of predation. Such models might help to elucidate the trade-offs for *B. brevitubus* females when ‘assessing’ resource availability and predation risk and ‘choosing’ their reproductive mode and sex allocation.

Split sex ratios and population structure

The distribution of sex ratios in *B. brevitubus* resembles that of the Australian gall-inducing thrips, *Kladothrips rugosus* (Kranz *et al.*, 2000). To the best of our knowledge, these are the only two species known to have split sex ratios as a result of a reproductive constraint (ovoviviparity in *B. brevitubus*, virginity in *K. rugosus*), with the offspring sex ratio produced by the unconstrained females (partly or fully oviparous and inseminated) as predicted by Godfray’s (1990) split sex ratio equation for panmictic populations. Of course, unlike virginity, ovoviviparous females are physiologically capable of switching to oviparity. However, while they retain eggs for ovoviviparous development, they are incapable of producing females.

Haga (1983) also found an unbiased sex ratio in *B. brevitubus* populations, and these were in Osaka, some 400 km directly southwest of our sites. However, we are cautious about deducing that the populations we studied were panmictic and the sex ratios were Fisherian (Fisher, 1930). First, we frequently observed aggregations of both sexes of adults

overwintering in the field and, in several cases, there was the remnant of an old egg mass on the same leaf. The females were inseminated and most of them had undeveloped oocytes (Fig. 2), indicating that they mated before overwintering and probably soon after eclosion. This suggests that the aggregations could have been siblings who had sib-mated. Second, we frequently observed sib-mating in the laboratory, both between individuals that had recently eclosed and those that had had previous mates. These points suggest that inbreeding and local mate competition (Hamilton, 1967) among brothers are likely, both of which would select for a female-biased sex ratio (Hamilton, 1967, 1972; Herre, 1985). Third, our data indicating competition between females for food or space suggest local resource competition (Clark, 1978) among females, which would select for a male-biased sex ratio. It is conceivable that these pressures are acting in tandem and could still result in a population-wide sex ratio. Such a 'balance' of inbreeding, local mate competition and local resource competition has been suggested to explain the unbiased sex ratio of soldiers in the inbred (Chapman *et al.*, 2000) eusocial galling thrips, *Kladothrips hamiltoni*, in which local mate competition and local resource competition were demonstrated (Kranz *et al.*, 1999, 2001). We are currently developing microsatellite markers to assess the population structure of *B. brevitubus*.

Predicting reproductive mode

Assuming that there are patches where mite predation, competition for food and egg-laying sites (local resource competition) and local mate competition operate, alone or together, we can begin to make specific predictions about what the trade-offs and preferred strategies are for reproductive females (Table 1). We also assume here that, other things being equal, oviparity is the cheaper strategy, because males guard egg masses, and that fecundity is higher using this mode. Based on these assumptions, we make the following predictions. A female should: (i) be oviparous if there are no constraints, because she has the advantage of paternal care and higher fecundity, and she can 'gamble' on the survivorship of her offspring; (ii) be oviparous if the only constraint is local mate competition, because only then can she produce females; (iii) be ovoviviparous if the only constraint is high predatory mite pressure, because producing eggs will lead to high mortality; (iv) be ovoviviparous if the only constraint is local resource competition, because then she does not require a whole leaf for laying an egg mass, and embryos can continue to develop while the female searches for a better feeding site for her offspring; (v) use a mixed strategy if there is mite pressure and local mate competition, because there is selection for both ovoviviparity and a female-biased sex ratio; and (vi) use a mixed strategy if there are mites, local mate competition and local resource competition. Scenarios (ii), (v) and (vi) are feasible in aggregations where sib-mating occurs. Scenario (vi) has the same constraints as for (v) but also has competition for food or egg-laying sites. These predictions are the bases for testable hypotheses that could elucidate how sex allocation and reproductive mode interact.

Social organization

Godfray and Grafen (1988), Godfray (1990) and Godfray and Hardy (1993) suggested that high levels of virginity in haplodiploid, panmictic populations select for a female bias in the offspring of mated (unconstrained) females, resulting in a population-wide unbiased sex ratio. Godfray (1990) extended this argument to any sperm limitation or constraint. They pointed out that such a bias could lower the threshold for the evolution of altruism and

sociality in haplodiploids. This is because, as Trivers and Hare (1976) explained, these females only gain fitness advantages through sib-rearing when they assist sisters more than they do brothers – a result of their higher relatedness to sisters than to either brothers or offspring. Our data suggest that *B. brevitubus* is probably subsocial (*sensu* Wilson, 1971). This is because (i) there was never more than one female guarding an egg mass, (ii) there was no evidence of communal egg laying, as the brood size on a leaf did not change significantly with the number of adult females, and (iii) there were no obvious differences in ovarian development between aggregating females. However, some idolothropines, such as *Sporothrips amplus* (Crespi, 1993) and *Anactinothrips gustaviae* (Keister and Strates, 1984), lay communal egg masses. The social status of the idolothropine group has been only superficially investigated, but determining the relationship between phylogenetic position, social status and sex allocation might help to elucidate evolutionary patterns of significance to further our understanding of social evolution.

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REFERENCES

- Abrams, P.A. 1993. Optimal traits when there are several costs – the interaction of mortality and energy costs in determining foraging behaviour. *Behav. Ecol.*, **4**: 246–253.
- Abrams, P.A. 1994. Should prey overestimate the risk of predation? *Am. Nat.*, **144**: 317–328.
- Alcock, J. 1998. *Animal Behaviour: An Evolutionary Approach*. Sunderland, MA: Sinauer Associates.
- Ananthakrishnan, T.N., Dhileepan, K. and Padmanaban, B. 1983a. Gut spore composition and influence of fungal host on the rate of mortality and post-embryonic development. *P. Indian A.S. – Anim. Sci.*, **92**: 11–17.
- Ananthakrishnan, T.N., Dhileepan, K. and Padmanaban, B. 1983b. Reproductive strategies and behavioural attributes in some sporophagous Idolothropinae (Tubulifera: Thysanoptera). *P. Indian A.S. – Anim. Sci.*, **92**: 95–108.
- Ananthakrishnan, T.N., Dhileepan, K., Padmanaban, B. and Suresh, G. 1984. Ecological interactions, species dynamics and reproductive biology of some mycophagous Thysanoptera. *Occas. Pub. Entomol. Res. Inst., Loyola College, Madras*, **5**: 1–37.
- Baur, B. 1994. Parental care in terrestrial gastropods. *Experientia*, **50**: 5–14.
- Breder, C.M. and Rosen, D.E. 1966. *Modes of Reproduction in Fishes*. New York: Natural History Press.
- Brown, J.S. 1999. Vigilance, patch use and habitat selection: foraging under predation risk. *Evol. Ecol. Res.*, **1**: 49–71.
- Chapman, T.W., Crespi, B.J., Kranz, B.D. and Schwarz, M.P. 2000. High relatedness and inbreeding at the origin of eusociality in gall-inducing thrips. *Proc. Natl. Acad. Sci. USA*, **97**: 648–650.
- Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian primate. *Science*, **210**: 163–165.
- Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Oxford: Oxford University Press.

- Crespi, B.J. 1988. Sex ratio selection in a bivoltine thrips. I. Conditional sex-ratio manipulation and fitness variation. *Evolution*, **42**: 1199–1211.
- Crespi, B.J. 1989. Facultative viviparity in a thrips. *Nature*, **337**: 357–358.
- Crespi, B.J. 1991. Heterozygosity in the haplodiploid Thysanoptera. *Evolution*, **45**: 458–464.
- Crespi, B.J. 1993. Sex ratio selection in Thysanoptera. In *Evolution and Diversity of Sex Ratio in Insects and Mites* (D.L. Wrensch and M. Ebbert, eds), pp. 214–234. New York: Chapman & Hall.
- Dhileepan, K. and Ananthakrishnan, T.N. 1986. The pleuropodium in the embryos of two species of viviparous sporophagous species of tubuliferan thrips (Thysanoptera: Insecta). *Curr. Sci. India*, **55**: 1005–1108.
- Dhileepan, K. and Ananthakrishnan, T.N. 1987. Impact of sex-limited and alary polymorphism on spermathecal diversity and reproductive behaviour in some mycophagous Tubulifera (Insecta: Thysanoptera). *Proc. Indian Natn. Sci. Acad. B*, **53**: 329–336.
- Dopazo, H.J. and Korenblum, M. 2000. Viviparity in *Salamandra salamandra* (Amphibia: Salamandridae): adaptation or exaptation? *Herpetologica*, **56**: 144–152.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press (1999).
- Godfray, H.C.J. 1990. The causes and consequences of constrained sex allocation in haplodiploid animals. *J. Evol. Biol.*, **3**: 3–17.
- Godfray, H.C.J. and Grafen, A. 1988. Unmatedness and the evolution of eusociality. *Am. Nat.*, **13**: 283–291.
- Godfray, H.C.J. and Hardy, I.C.W. 1993. Sex ratio and virginity in haplodiploid insects. In *Evolution and Diversity of Sex Ratio in Insects and Mites* (D.L. Wrensch and M. Ebbert, eds), pp. 402–417. New York: Chapman & Hall.
- Haga, K. 1972. On the Thysanoptera inhabiting dead leaves and dead branches. *Ann. Rep. Tennoji High School, Osaka Kyoiku Univ.*, **13**: 100–113 (in Japanese).
- Haga, K. 1974. Postembryonic development of the megathripine species, *Bactridothrips brevitubus* Takashi (Thysanoptera). *Sugadaira Biol. Lab. Tokyo Kyoiko Univ.*, **26**: 11–32.
- Haga, K. 1975. Female reproductive system of megathripine species, *Bactridothrips brevitubus* (Thysanoptera: Insecta). *Sugadaira Biol. Lab. Tokyo Kyoiko Univ.*, **32**: 13–24.
- Haga, K. 1980. The life history of *Bactridothrips brevitubus* (Takahashi) (Idolothripinae). In *Proceedings of the XVI International Conference of Entomology: Thysanopterists Workshop*, pp. 1–8. Kyoto: ICE.
- Haga, K. 1983. Supplementary notes on the ovoviviparity of *Bactrothrips brevitubus* (Insecta: Thysanoptera). *Proc. Arthropod. Embryol. Soc. Jpn.*, **9**: 21.
- Haga, K. and Okajima, S. 1989. A taxonomic study of the genus *Bactrothrips* Karny (Thysanoptera, Phlaeothripidae) from Japan. *Bull. Sugadaira Montane Res. Cen.*, **10**: 1–23.
- Hagan, H.R. 1951. *Embryology of the Viviparous Insects*. New York: Ronald Press.
- Hamilton, W.D. 1967. Extraordinary sex ratios. *Science*, **156**: 477–488.
- Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.*, **3**: 193–232.
- Heller, J., Sivan, N. and Hodgson, A.N. 1997. Reproductive biology and population dynamics of an ovoviviparous land snail, *Lauria cyclindracea* (Pupillidae). *J. Zool.*, **243**: 263–280.
- Heming, B.S. 1991. Order Thysanoptera. In *Immature Insects*, Vol. 2 (F.W. Stehr, ed.), pp. 1–21. Dubuque, IA: Kendall/Hunt.
- Herre, E.A. 1985. Sex ratio adjustment in fig wasps. *Science*, **228**: 896–898.
- Hughes, R.N. 1995. Resource allocation, demography and the radiation of life histories in rough periwinkles (Gastropoda). *Hydrobiologia*, **309**: 1–14.
- Keister, A.R. and Strates, E. 1984. Social behaviour in a thrips from Panama. *J. Nat. Hist.*, **18**: 303–314.
- Kranz, B.D., Schwarz, M.P., Mound, L.A. and Crespi, B.J. 1999. Social biology and sex ratios of the eusocial gall-inducing thrips *Kladothrips hamiltoni*. *Ecol. Entomol.*, **24**: 432–442.

- Kranz, B.D., Schwarz, M.P., Giles, L.C. and Crespi, B.J. 2000. Split sex ratios and virginity in a gall-inducing thrips. *J. Evol. Biol.*, **13**: 700–706.
- Kranz, B.D., Chapman, T.W., Crespi, B.J. and Schwarz, M.P. 2001. Social biology and sex ratios in the gall-inducing thrips, *Oncothrips waterhousei* and *Oncothrips habrus*. *Insect. Soc.*, **48**: 315–323.
- Kucharzyk, H. 1993. Observations on the phenomenon of viviparity in thrips species living in xerothermic grasslands in Poland. In *Advances in Thysanopterology*, Vol. 4. (J.S. Bhatti, ed.), pp. 259–265. New Delhi: Scientia Publishing.
- Kudo, S., Ishibashi, E. and Makino, S. 1995. Reproductive and subsocial behaviour in the ovoviviparous leaf beetle *Gonioctena sibirica* (Coleoptera: Chrysomelidae). *Ecol. Entomol.*, **20**: 367–373.
- Lewis, T. 1973. *Thrips: Their Biology, Ecology and Economic Importance*. London: Academic Press.
- Macfarlane, R.B., Norton, E.C. and Bowers, M.J. 1993. Lipid dynamics in relation to the annual reproductive cycle in yellowtail rockfish (*Sebastes flavidus*). *Can. J. Fish. Aquat. Sci.*, **50**: 391–401.
- Meier, R., Kotrba, M. and Ferrar, P. 1999. Ovoviviparity and viviparity in the Diptera. *Biol. Rev.*, **74**: 199–258.
- Mound, L.A. and Palmer, J.M. 1983. The generic and tribal classification of spore-feeding Thysanoptera (Phlaeothripidae: Idolothripinae). *Bull. Br. Mus. Nat. Hist. (Ent.)*, **46**: 1–174.
- Neill, W.T. 1964. Viviparity in snakes: some ecological and zoogeographical considerations. *Am. Nat.*, **48**: 35–55.
- Roth, L.M. 1989. *Sliferia*, a new ovoviviparous cockroach genus (Blattellidae) and the evolution of ovoviviparity in Blattaria (Dictyoptera). *Proc. Entomol. Soc. Wash.*, **91**: 441–451.
- Shine, R. 1985. The evolution of viviparity in reptiles: an ecological analysis. In *Biology of Reptiles*, Vol. 15 (C. Gans and F. Billet, eds), pp. 604–694. New York: Wiley.
- Shine, R. and Bull, J.J. 1989. The evolution of live-bearing in lizards and snakes. *Am. Nat.*, **113**: 905–923.
- Stannard, L.J. 1968. The thrips, or Thysanoptera of Illinois. *B. Illinois St. Nat. Hist. Surv.*, **29**: 215–552.
- Tallamy, D.W. 2000. Sexual selection and the evolution of exclusive paternal care in arthropods. *Anim. Behav.*, **60**: 559–567.
- Trivers, R.L. and Hare, H. 1976. Haplodiploidy and the evolution of social insects. *Science*, **191**: 249–264.
- Wake, M.H. 1993. Evolution of oviductal gestation in amphibians. *J. Exp. Zool.*, **266**: 394–413.
- Wells, K.D. 1981. Parental behavior of male and female frogs. In *Natural Selection and Social Behavior* (R.D. Alexander and D.W. Tinkle, eds), pp. 184–197. New York: Chiron Press.
- Wilson, E.O. 1971. *The Insect Societies*. Cambridge, MA: Harvard University Press.
- Wourms, J.P. 1977. Reproduction and development in chondrichthyan fishes. *Am. Zool.*, **17**: 379–410.