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Female-biased sex allocation in wild populations of the eriosomatine aphid *Prociphilus oriens*: Local mate competition or transgenerational effects of maternal investment?

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Abstract Several aphid species exhibit female-biased sex allocation. Local mate competition (LMC) has been postulated to be the evolutionary factor of the female-biased sex allocation. We estimated individual sex allocation in the eriosomatine aphid *Prociphilus oriens* and explained the observed pattern of sex allocation based on a hypothesis other than LMC. On the basis of the relationship between maternal body size and brood size, we estimated the cost of producing a female to be 1.85 times the cost of producing a male. The population-wide allocation to males was 22%-24%. Winged mothers exhibited a large variation in the number of male and female embryos they had, including 23%-30% of winged mothers producing only female embryos. There was polymorphism in the sex-ratio expression. Thus, the constant male hypothesis assuming LMC was not supported. Winged mothers that produced an all-female brood contained larger female embryos than did mothers that produced a bisexual brood. Previous studies have indicated that a large sexual female produces a single large egg, which hatches into a first-instar larva containing a larger amount of gonads. Thus, in eriosomatine aphids, maternal investment in daughters directly affects the potential fecundity of granddaughters, whereas investment in sons does not. We propose a hypothesis that higher fitness returns from maternal investment in daughters than in sons may have primarily led to the evolution of highly female-biased sex allocation in *P. oriens*.

Keywords Eriosomatinae · Parental investment · Parthenogenesis · Patch · Polymorphism · Sex ratio

Introduction

In most insects and mites, sex allocation by mothers is affected by the structure of the population (Kirkendall 1993; Moran 1993; Foster 2002; Sabelis et al. 2002) and adjusted in some taxa according to the conditions the mothers confront in the breeding patch (West 2009). Population structure is affected by several factors including the spatial distribution and persistence of resources, the mobility of males and females. Thus, for the understanding of sex allocation in a species, it is necessary to focus on the spatiotemporal distribution of resources, and the mobility and competition of the focal species. In a randomly mating population, maternal investment on sons and daughters is expected to be equal (Fisher 1930). This is because in panmictic populations competition among males for mates and competition among females for resources will be global and balanced with each other. However, if competition among males is local, economizing on investment in sons will increase maternal fitness by reducing competition among sons (Hamilton 1967). This situation occurs when a population is composed of patches where a small number of foundress mothers reproduce and their progeny mate among themselves. In this case, local mate competition (LMC) among sons leads to female-biased sex allocation (Hamilton 1967). Sex allocation is also affected by the resource condition of mothers (Trivers and Willard 1973) although little attention has been paid to this factor in insects. However, maternal conditions could be an important factor affecting sex allocation even in insects if increased maternal investment in one sex has a larger effect in the return from that sex.

Aphid species of Eriosomatinae (Hemiptera, Aphididae) exhibit female-biased sex allocation, with a few exceptions (Tanaka 1961; Janiszewska-Chichocka 1969; Yamaguchi 1985; Kurosu and Aoki 1991; Foster and Benton 1992; Moran 1993; Wool and Sulami 2001; Akimoto and Yamaguchi 2004). This tendency is accounted for in

terms of LMC under the condition of foundress mothers containing variable amounts of resources (Yamaguchi 1985; Frank 1987a,b; Kindlmann and Dixon 1989). These models assuming an evolutionary stable strategy (ESS) predict that mothers in a patch should produce a constant number of sons (constant male hypothesis) and that mothers with more resources than a threshold level should produce more daughters. Yamaguchi (1985) first verified the male constant hypothesis by using the eriosomatine aphid *Prociphilus oriens* Mordvilko, 1935; all *P. oriens* mothers produced 4 male embryos and various numbers of female embryos in their abdomen. Although predictions from the models have been generally supported by the data, these models are based on some unrealistic assumptions; that is, mothers are assumed to completely know the resource levels of other mothers in the same patch (May and Seger 1985; Kindlmann and Dixon 1989; Stubblefield and Seger 1990; Foster and Benton 1992; West 2009). Stubblefield and Seger (1990) relaxed some conditions of these models and reexamined an optimal sex allocation by using ESS models; their revised model predicts that a constant number of males is not observed, but that mothers produce an increasing number of males with larger amounts of resources. However, across some eriosomatine species, a considerable proportion of mothers produce only female embryos (Moran 1993; Akimoto and Yamaguchi 2004). Our preliminary study also confirmed that in *P. oriens*, mothers producing an all-female brood accounted for a high proportion in some years. No ESS model has predicted the occurrence of such mothers that produce only daughters. This observation motivated us to reexamine the sex allocation in *P. oriens* and investigate the causal mechanism underlying the observed sex ratios.

Prociphilus oriens is a host-alternating aphid that is associated with the primary host *Fraxinus mandshurica* Ruprecht and the secondary host *Abies sachalinensis* Masters (Blackman and Eastop 1994). The first parthenogenetic generation hatches from overwintered eggs early in spring, and parthenogenetically produces the second

generation on the twigs of *Fraxinus mandshurica* in late May. The second generation develops into winged females and migrates to the *Abies sachalinensis* trees, on which they larviposit (the third generation) parthenogenetically. The first-instar larvae move from *A. sachalinensis* twigs to the underground parts where they grow under the attendance of mutualistic ants and develop into wingless adults. Wingless generations repeat parthenogenetic reproduction on the roots of *Abies sachalinensis* from early summer to autumn. In early to late October, a winged generation emerges on *A. sachalinensis* and migrates to *F. mandshurica*. On calm days in mid October, a large mass of winged females flies around *Fraxinus mandshurica* trees. On *F. mandshurica* trunks, winged females aggregate, forming masses of a few to tens mothers and produce males and sexual females simultaneously (S. Akimoto, unpublished data). This breeding characteristic facilitates local mate competition among males of the same brood (Yamaguchi 1985). These sexuals are dwarfish, molt 4 times without feeding, and mature sexually in 1 week. The abdomen of each sexual female contains only a single large egg (Heie 1980), which is deposited in a bark crevice approximately 5 days after copulation (Akimoto 2006). The mating behavior and breeding system of *P. oriens* are almost the same as those of *Pemphigus spyrothecae* described by Foster and Benton (1992). Eggs overwinter in the crevices of the trunk and hatch in late April before the bud burst of *F. mandshurica*.

In general, it is difficult to evaluate the amount of maternal investment in male and female offspring if maternal investment includes expenditures for egg production, foraging, food provisioning and guarding against natural enemies. However, eriosomatine aphids have an advantage in this respect because the maternal generation (autumnal winged aphids called sexuparae) parthenogenetically produces male and female embryos in their ovarioles and provides them with all nutrients required for their development (Miyazaki 1987; Moran 1993). Males and sexual females, called sexuals,

develop fully within their mother and do not feed after they are born viviparously. Thus, maternal investment in eriosomatine aphids is completed before larviposition, and the sex allocation can be estimated by counting the number of male and female embryos within the mother and by evaluating the costs incurred during their development (Moran 1993; Foster 2002).

The second advantage of evaluating sex allocation in eriosomatine aphids is that the winged maternal generation swarms in a large mass around the primary host plants; therefore, it is easy to collect a number of individuals randomly for evaluating the population sex allocation. The evaluation of sex allocation by mothers requires the assessment of the production costs of male and female embryos. However, it is not obvious whether the weight, volume, area, or caloric content of the embryos is appropriate for the maternal investment index. In a study of sex allocation in *P. oriens*, Yamaguchi (1985) estimated the volume of a female embryo to be 3.0 times that of a male embryo. In the present study, we propose a new method for evaluating the relative cost of producing females of *P. oriens* and determine the exact pattern of sex allocation by wild populations of *P. oriens*.

Another issue in assessing the population sex allocation of eriosomatine aphids by dissection of winged mothers is the possible variation in sex allocation among mothers swarming on different days or among those appearing in different microhabitats. In order to understand the evolution of sex allocation in eriosomatine aphids, it is necessary to know the spatiotemporal variation in the sex ratios of local populations.

Therefore, the specific objectives of the present study are (1) to assess the number of male and female embryos produced by individual winged mothers of *P. oriens* in wild populations, (2) to examine the spatiotemporal variation in population sex ratios, and (3) to evaluate the sex allocation of wild populations by estimating the relative cost of producing a female versus that of producing a male embryo. On the basis of these data,

we propose a new hypothesis to account for the highly female-biased sex ratio in eriosomatine aphids.

Materials and methods

Collection of winged mothers and measurements

Swarming winged females were collected in Iwamizawa, Hokkaido, northern Japan, on October 17, 1998, and in Sapporo, Hokkaido on October 7 and 11, 2002 by using an insect net. The collected winged females were immediately preserved in vials of 80% ethanol and maintained until the number of the sexuals was counted. For the investigation of the sex ratios, we used 270 and 385 winged mothers, respectively, for the 1998 and 2002 samples. These mothers were dissected under a binocular microscope with forceps and fine insect pins, and the number of male and female embryos in their abdomens was counted. Males and sexual females are easily distinguished by the size and shape. For the 1998 samples, the forewings of all the 270 winged mothers were cut off and mounted on glass slides by using Hoyer's medium for measurements. The forewing lengths were used as an index of the body size of winged mothers. Of the 2002 samples collected in Sapporo on October 7, 35 mothers that produced 4 males and any number of females and 28 mothers that produced females alone were randomly selected, and all the sexuals from these winged mothers were mounted on glass slides for image analysis. Male and female embryos from each mother were mounted on 1 glass slide together with the forewings of their mother, and the mounted slides were maintained at 60°C in an incubator for 4 hours. The images of mounted sexuals and forewings were captured into a video camera (Victor TK-C1380) installed on a binocular microscope (Olympus SZX12) and transferred to a computer.

For the evaluation of the production costs of male and female embryos, their dry mass was weighed. For this purpose, we used sexuals taken from 28 winged mothers collected in Iwamizawa in 2009. Female or male embryos from each mother were dried in an oven and weighed for each sex together on a microbalance (Sartorius SE2), and the average dry weight was calculated for each of female and male embryos.

The 2002 samples consisted of 3 groups of winged mothers: (1) those collected from the campus of Hokkaido University, Sapporo, on October 7; (2) those collected from the same location on October 11, and (3) those collected from the Asahiyama Park, Sapporo, 4.0 km from the campus on October 7. The city area separated the two points. Thus, the temporal variation in the sex ratio was examined by comparing the first and second groups of winged mothers, and spatial variation was examined by comparing the first and third groups of winged mothers.

Hypothesis and statistics

Winged mothers of this species do not feed after emergence and produce sexuals by using resources that they acquire during their development. We postulate that winged mothers of the same size could invest almost the same amount of resources in their sexual offspring, although the number of males and females may vary depending on their developmental environments. We also hypothesize that the larger the winged mothers, the larger the amount of resources they can invest in sexual offspring. In particular, we focus on 2 types of winged mothers (see Results) for assessing the costs incurred in male and female production: (1) winged mothers producing 4 males and any number of females (4-male-producing mothers), and (2) those producing only females (female-producing mothers). Our basic hypothesis is that if there is difference in the female number between the 2 types of winged mothers, this difference (*D*) comes from

the cost of producing 4 males in 4-male-producing mothers. Therefore, we can assume that the cost of producing *D* females is equivalent to the cost of producing 4 males. Based on this hypothesis, the relative cost of producing a female versus male embryo was calculated separately for the 1998 and 2002 samples. We estimated the mean *D* by adjusting the size of winged mothers. To evaluate the difference *D*, we performed ANCOVA, in which the number of female embryos was treated as the response variable, the 2 mother groups (female-producing and 4-male-producing mothers) as the independent variable, and the forewing lengths of winged mothers as the covariate. In this analysis, we calculated the least square mean of female numbers for the 2 mother groups.

The relative cost was estimated by another method: we captured computer images of all embryos from each mother and compared the area of male and female embryos by using image analysis. The area of the image of each male or female embryo was measured using the software NIH image ver. 6.2 (http://rsbweb.nih.gov/nih-image/) using the 2002 samples after the original images were transformed into binary data (black and white pixels) at a threshold density. The results from the 2 methods were compared to obtain a consensus. On the basis of the obtained relative cost of producing females, we evaluated the sex allocation in wild populations.

The total image area of male and female embryos from each mother was calculated and regarded as the total investment of the mother. The total investment was analyzed by ANCOVA, in which the 2 mother groups were treated as the independent variable and forewing length as the covariate. The interaction between the mother groups and forewing length was also included in the model.

The relationship between the forewing length of winged mothers and the mean size of their male or female embryos was examined using linear regression.

Results

Population sex ratios

The total number of males and females in the 1998 samples was 669 and 1296, respectively, and 1044 and 1771, respectively, in the 2002 samples. The numerical female sex ratio was 0.660 and 0.629, respectively, for the 1998 and 2002 samples. These ratios were more female-biased than the ratio of 0.5 obtained by Yamaguchi (1985) (for the 1998 data, $\chi^2 = 95.0$, $df = 1$, $P < 0.0001$, for the 2002 data, $\chi^2 = 72.4$, $df = 1$, $P < 0.0001$). When winged mothers were categorized with respect to the number of males they had in their abdomen, there were 2 main categories: 4-male-producing mothers and female-producing mothers (Table 1). The number of female embryos per mother significantly decreased with an increasing number of male embryos per mother in both years (linear regression for the 1998 samples, $df = 1, 268$, $t = -8.29$, $P < 0.0001$; for the 2002 samples, $df = 1, 383$, $t = -13.65$, $P < 0.0001$). However, no significant differences were found in the forewing length of winged mothers among the mother categories (ANOVA, $df = 4, 265$, $F = 1.65$, $P = 0.162$).

For the 2002 samples, variation in the sex ratio between different swarming days in the same place and between different microhabitats on the same day was examined (Table 2). There were no significant differences in the frequencies of the mother categories among the 3 samples (G test, $df = 4$, $G = 3.87$, $P = 0.424$). On the other hand, there was a significant difference in the sex ratio between samples collected from different microhabitats, 4.0 km apart, on the same day ($df = 1$, $G = 20.68$, $P < 0.0001$), but no significant difference was found in the sex ratio between different swarming days at the same place (G test, $df = 1$, $G = 2.614$, $P = 0.106$).

Relative cost of producing female embryos

The results of ANCOVA indicated that female-producing mothers had a larger number of female embryos than did 4-male-producing mothers (for the 1998 samples, $df = 1$, $F = 203.5$, $P < 0.0001$; for the 2002 samples, $df = 1$, $F = 74.4$, $P < 0.0001$). The difference between the least square means of female numbers (*D* in Fig. 1) was 2.161 for the 1998 samples and 2.148 for the 2002 samples. If this contrast in female number was caused by the cost of producing 4 males, the cost of producing 1 female embryo was estimated to be 1.851 and 1.863 times as much as the cost of producing 1 male embryo for the 1998 and 2002 samples, respectively.

The mean area of female embryos from a mother was 1.825 times as large as the mean area of male embryos, and this value was close to the values estimated as the relative cost of producing female embryos. The total investment per mother (the total area of sexuals) increased with the forewing length of the winged mothers for both mother groups (Fig. 2, linear regression for female-producing mothers, $df = 1, 26$, $t = 16.32$, $P < 0.0001$; for 4-male-producing mothers, $df = 1, 33$, $t = 15.17$, $P < 0.0001$); there was no significant interaction between the mother groups and the forewing length (ANCOVA, $df = 1$, $F = 0.067$, $P = 0.797$). Furthermore, there was no significant difference between the intercepts of the regression lines (ANCOVA, $df = 1$, $F = 0.50$, $P = 0.482$). These results indicate that a common regression line can be applied to the 2 mother groups (Fig. 2).

The dry weight of females (the mean weight of 26 broods) was 3.60 times that of males (the mean weight of 16 broods).

Size of male and female embryos

There was no significant relationship between the forewing length of a winged mother and the mean area of female embryos from the mother for both mother categories (Fig. 3a; linear regression for female-producing mothers, df = 1, 26, t = -0.78, P = 0.440; for 4-male-producing mothers, df = 1, 31 , t = 0.94, P = 0.353). However, the mean area of female embryos in female-producing mothers was significantly larger than that of female embryos in 4 male-producing mothers (Fig. 3a; ANOVA, df = 1, 59, F = 5.00, P = 0.029). On the other hand, in 4-male-producing mothers, the mean area of male embryos from a mother decreased with increasing maternal forewing length (Fig. 3a; linear regression, df = 1, 34, t = -3.46, P = 0.0015). As a result, with increasing maternal forewing length, the total area of female embryos (total investment in females) increased in both mother categories (Fig. 3b; linear regression for female-producing mothers, df = 1, 26, t = 16.32, P < 0.0001; for 4-male-producing mothers, df = 1, 33, t = 16.16, P < 0.0001), but the total area of male embryos (total investment in males) decreased (linear regression for 4-male-producing mothers, df = 1, 33, t = -3.37, P = 0.0019).

The relationship between the size of the mothers (forewing length) and percentage allocation to males was demonstrated for the 1998 samples (Fig. 4), for which the forewing lengths of all the mothers were measured. There was an overall tendency for percentage allocation to males to decrease with increasing forewing length of the mothers (linear regression, df = 1, 268, t = -10.30, P < 0.0001); however, this tendency was not rigid (R^2 = 0.284) because of the involvement of female-producing mothers.

Discussion

The results of our study indicate that contrary to previous studies, winged mothers of *P. oriens* produce various numbers of males and females. Although mothers producing 4

males were still the main type in the population as observed by Yamaguchi (1985), we observed frequent occurrences of other types, including a high frequency of mothers producing females only. This finding suggest that *P. oriens* exhibits polymorphism in the sex-ratio expression. Similarly, Moran (1993) reported that for 2 American *Prociphilus* species, *P. corrugatans* and *P. fraxinifolii,* winged mothers exhibited a large variation in the sex ratio, including those producing unisexual broods. The coexistence of mothers producing an all-female brood and those producing a bisexual brood in a population has not been reported in other animal species as far as we know. However, we can find similar examples in plant species known as gynodioecious: species in which hermaphrodite plants coexist with plants bearing only female flowers (Charlesworth and Charlesworth 1978). This parallel phenomenon suggests that similar selective pressures may be acting on the sex expression of *P. oriens* populations and some plant species.

Among eriosomatine aphid species, the constant male hypothesis is supported by studies on *Tetraneura nigriabdominalis* (Tanaka 1961), *Pemphigus spyrothecae* (Foster and Benton 1992), *Kaltenbachiella elsholtriae* (Kurosu and Aoki 1991), and *Eriosoma crataegi* (Moran 1993), in addition to *Prociphilus oriens* (Yamaguchi 1985). On the other hand, a considerably high proportion of female-producing mothers was recorded in some eriosomatine species. For example, the winged mothers of *Tetraneura sorini* have no tendency to produce a constant number of males, with 48.6% of them containing sexual females alone (Akimoto and Yamaguchi 2004). Rearing of clones on the secondary host indicated that 9 clones of *Tetraneura* sp. O produced winged mothers that contained a constant male number (5 or 6 males), while 1 clone produced winged mothers that contained sexual females only (Akimoto and Yamaguchi 2004). In their study on the sex ratios of 9 eriosomatine and hormaphidine species, Kurosu and Aoki (1991) indicated that in holocyclic species (host-alternating and sexual species), the male constant rule is generally supported, while in species in which holocyclic and

anholocyclic (non-host-alternating and asexual) strains coexist, the male constant rule is violated probably because of immigration of winged mothers from anholocyclic strains.

Anholocyclic strains can reproduce parthenogenetically on the secondary host from year to year but produce winged mothers that migrate to the primary host in the autumn. There is no information about the biological function of such winged mothers. Because no natural selection is acting on the sexual traits of anholocyclic strains, Kurosu and Aoki (1991) hypothesized that some genes that deviate the sex ratio from the male constant rule may have accumulated in anholocyclic strains. If their hypothesis were correct, violation of the male constant rule would suggest that winged mothers derived from anholocyclic strains are included in the samples. However, we cannot rule out the possibility that winged mothers with a varying number of males have evolved in a holocyclic population. At least in Hokkaido, northern Japan, where winter is very severe, *P. oriens* consists of only holocyclic strains. In future studies, it will be critically important to examine whether the difference in the sex ratio of *P. oriens* can be attributed to the differences between localities and/or the collection years.

The present study evaluated the range of spatiotemporal variation in sex ratios. The sex ratio was similar between samples from different swarming days, providing evidence for the uniformity of populations. Foster (2002) predicted that winged mothers flying later to a patch may have more female-biased broods because female offspring are likely to mate with old males produced by early-coming mothers of the same clone. However, our data did not support this hypothesis. On the other hand, the sex ratio was affected by the differences in microhabitats only 4.0 km apart. These data suggest that if collection of winged mothers is conducted in places more distant from each other, we could observe large variations in the sex ratio. Therefore, in future studies, it is necessary to evaluate both the geographic variation and long-term fluctuations in the sex ratio.

The present study estimated the cost of producing females to be 1.83–1.86 times higher than the cost of producing males. These estimates were much lower than those based on the volume (3.0 times, Yamaguchi 1985) or dry mass (3.6 times) of the sexuals. Our estimate postulates that both female-producing mothers and 4-male-producing mothers invest the same amount of resources in their offspring if their body size is equal. In contrast, if the cost of producing females is 3 times or more the cost of producing males, then female-producing mothers will invest much more resources in the offspring than do 4-male-producing mothers of the same size. However, this conclusion is difficult to accept because both categories of mothers emerged from the same secondary host colonies (S. Akimoto, unpublished data). Thus, if the median (1.85) was used as the relative cost of producing females, the population-wide investment in females would be 78.2% (21.8% in males) in the 1998 samples and 75.8% (24.2% in males) in the 2002 samples.

Maternal body size affected the sex allocation pattern. If we focus on 4-male-producing mothers, as they become larger, they invest more in females but less in males with decreasing body size of individual males. This fact suggests that depending on the nutritional conditions on the secondary host, individual mothers can adjust their sex allocation. When winged mothers grow in poor nutritional conditions or in crowded conditions, they will tend to invest more resources in respective males. However, differences in maternal body size were not a factor in determining the number of males the mothers developed in their abdomens. It is not clear what developmental cues determine the number of males within winged mothers.

A markedly female-biased sex ratio is commonly observed among aphids, even in host-alternating species, where outbreeding is certain (Ward and Wellings 1994; Dixon 1998; Miller and Aviles 2000). Female-biased sex allocation in aphids has been explained in terms of LMC by many authors, but there is no compelling and

independent evidence for LMC in aphids. As several authors have pointed out (Kindlmann and Dixon 1989, Stubblefield and Seger 1990, Foster 2002, West 2009), the brood sex ratio in an eriosomatine mother is determined during her development before arrival at a patch on the primary host. Hence, winged mothers cannot fine-tune their offspring sex ratios after they arrive at a patch. Nevertheless, Yamaguchi's model (1985) requires that each mother has to know the mean parental investment (PI) of the foundresses as well as the mean foundress number (n) in the patch because she has to determine her sex ratio by comparing her own PI with the mean population PI (Kindlmann and Dixon 1989, Stubblefield and Seger 1990, Dagg and Vidal 2004). The ESS sex ratio m_i for the ith mother in Yamaguchi's model is represented by $m_i = (P/P_i)*(n\text{-}1)/2n$, where P is the mean PI of the foundress mothers in the patch, P_i is the PI of the ith mother, and n is the number of foundresses in the patch. Yamaguchi (1985) postulated that *P. oriens* form a small patch of, on an average, 4 winged mothers. For such a small patch, we can expect local mate competition and inbreeding. However, because the size of foundress populations appears to fluctuate from year to year depending on the population density, it is difficult to assume that mothers always produce sexuals in small patches.

Akimoto's (2006) breeding experiments indicate that clonal self-fertilization, which is predicted in small patches, is rare in wild populations of *P. oriens* even though a low level of inbreeding is possible. Kindlmann and Dixon (1989) modified Yamaguchi's model and assumed a negative correlation between the overall density of winged mothers in a year and the mean body size of winged mothers (and PI). Assuming this relationship, they proposed that winged mothers can predict the mean PI and mean foundress number in a patch; thus the LMC model can be revived. However, the validity of this assumption has not been confirmed (Foster 2002), and it is necessary to examine if LMC occurs each year. Miller and Aviles (2000) explained female-biased sex ratio

observed in *Tamalia coweni* by the Trivers-Willard (1973) hypothesis of condition-dependent sex allocation. They suggest that if *T. coweni* clones that develop in favorable environmental conditions can produce larger sexual females with higher potential fecundity, clones in favorable conditions would allocate more resources to sexual females than to males.

Eriosomatine aphids are peculiar in that mothers invest different amounts of resources in male and female embryos. For hermaphrodites including aphid clones (disaggregated hermaphrodites; Foster 2002), an optimal sex allocation should deviate from 1:1 if male and female offspring have different patterns of fitness returns to a given level of investment (Charnov 1982; Brunet 1992). If a mother's reproductive output through sons and daughters is represented as x^a and $(1\text{-}x)^b$, respectively, to the investment in sons (x) and daughters ($1\text{-}x$), the ESS sex allocation to males x' is represented as $x' = a/(a+b)$ (Seger and Stubblefield 2002). In particular, if the increase in fitness returning to unit investment is greater in daughters than in sons, an optimal sex allocation is expected to be female-biased. In eriosomatine aphids, investment in daughters could result in a greater fitness return because it can affect the potential fecundity of granddaughters. On the other hand, investment in sons does not influence the fecundity of granddaughters. Given that a mother's fitness increases linearly with investment in sons ($a = 1$) in an equilibrium population with an allocation of 22% to 24% to males, then fitness gain through investment in daughters is estimated to increase at a rate of investment to the power (b) of 3.1 to 3.6.

In eriosomatine aphids, the size of sexual females is positively correlated with the size of first-instar larvae that emerge the next spring (Akimoto and Yamaguchi 2004). Each sexual female has only a single egg, which occupies most of the female, and from the egg hatches a first instar, which moves to a developing leaf on the host plant and induces a leaf gall to larviposit parthenogenetically. First instars hatching from larger

eggs probably have higher fecundity in adulthood because they have a disproportionally large amount of gonads; the allometry of larval body parts on egg size is negative, whereas the allometry of larval gonads on egg size is positive with an allometry coefficient of 1.5 (Akimoto 2006, Fig. 1 for first instars derived from outbreeding). This result suggests that enhanced maternal investment in sexual females results in larger eggs and higher fecundity in granddaughters. In contrast, investment in males may not yield such a high fitness return because male body size is unlikely to affect his reproductive success. Males in eriosomatine aphids less intensively compete with one another for access to females (Foster and Benton 1992); on the back of females, males often try to push the rivals away from the females. However, there are no data that larger males are advantageous in this weak level of competition. Reproductive characteristics in eriosomatine aphids, i.e., a transgenerational effect of investment in daughters, have been neglected as an explanation for female-biased sex ratio. On the other hand, in ordinary insects including Hymenoptera, egg size is determined at a certain size before fertilization, and thus only the number of eggs allocated to each sex controls sex allocation. Therefore, in other insects, it is unlikely that maternal investment in female eggs directly affects the fecundity of daughters.

The transgenerational effect hypothesis for female-biased sex allocation is similar to Trivers-Willard (1973) hypothesis in postulating that mothers invest more to the sex that derives higher reproductive success from a given level of investment. However, narrow-sense Trivers-Willard hypothesis focuses on the situation where the conditions of individual mothers affect the size of newborn males, which in turn determines future reproductive success. Thus, this hypothesis accounts for adaptive sex allocation by individual mothers. By contrast, since *P. oriens* mothers produces the same-sized daughters in each mother category, this hypothesis cannot apply to each mother's adaptive sex allocation but to population-level sex allocation. Furthermore, the

transgenerational effect hypothesis would point to the advantage of mothers producing an all-female brood. Because these mothers can give birth to larger females, on an average, this type of mothers will enjoy higher reproductive success in the granddaughter generation. Although there is no information about the genetic basis of mothers producing an all-female brood, this reproductive advantage may have led to increasing proportions of this type of mothers. The transgenerational effect of maternal investment would have affected the evolution of sex allocation independently of LMC. However, the effect of LMC cannot be neglected in years when the population density is low enough to permit clonal self-fertilization (Ward 1991). It is also necessary to point out that the examples of highly female-biased sex allocation and variable male numbers are seen in such eriosomatine species that produce large-sized sexual females relative to the mothers (Akimoto and Yamaguchi 2004). Therefore, selective pressures for producing large sexual females appear to have incidentally evolved female-biased sex allocation and variable male numbers per mother.

In future studies, it is necessary to describe the diversity of sex allocation patterns among aphid species and elucidate evolutionary trends in sex allocation throughout the phylogeny of an aphid group for better understanding of the evolution of sex allocation in aphids.

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Figure legends

Fig. 1. The relationship between the forewing length of a winged mother and the number of female embryos that the mother contains. For two groups of winged mothers (female-producing and 4-male-producing mothers) from the 1998 samples, regression lines with the same slope were applied to evaluate the difference (*D*) between the intercepts.

Fig. 2. The relationship between the forewing length of a winged mother and the total area (total investment) of all male and female embryos from the mother. Two groups of winged mothers (female-producing and 4-male-producing mothers) are indicated. Regression lines were separately applied to the two mother groups. There was no significant difference in the slope or the intercept between the two regression lines. Based on the 2002 samples.

Fig. 3. The relationship between the forewing length of a winged mother and the mean area or total area of each sex's embryos from the mother. Two groups of winged mothers (female-producing and 4-male-producing mothers) are shown from the 2002 samples. (a) mean area of male or female embryos, (b) total area of male or female embryos.

Fig. 4. The relationship between the forewing length of a winged mother and percentage allocation to males per mother in the 1998 samples.

Table 1. The total number of winged mothers, mean number of females per mother and mean forewing length of mothers in each mother category in the Iwamizawa and Sapporo populations

Mother category (Number of male embryos per mother)	Iwamizawa population 1998			Sapporo population 2002	
	Number of mothers (%)	Mean number of female embryos	Mean forewing length of mothers (mm)	Number of mothers (%)	Mean number of female embryos
0	82 (30.4)	6.57	4.02	90 (23.4)	7.22
1	12 (4.4)	5.50	3.91	17 (4.4)	5.47
2	9 (3.3)	5.89	4.04	28 (7.3)	4.42
3	29 (10.7)	4.69	3.98	31 (8.1)	4.16
4	138 (51.1)	3.64	3.90	219 (56.9)	3.54

Table 2. The total number of winged mothers in each mother category and sexuals in three subpopulations of the Sapporo population in 2002. The categories of mothers with 1 to 3 males were pooled because of the small sample sizes

Mother category (Number of male embryos per mother)	Number of aphids (%)		
	Hokkaido Univ., Sapporo, Oct. 7	Hokkaido Univ., Sapporo, Oct. 11	Asahiyama Park, Sapporo, Oct. 7
0	35 (19.4)	28 (27.1)	27 (26.5)
1 to 3	36 (20.0)	23 (22.3)	19 (18.6)
4	109 (60.6)	52 (50.5)	56 (54.9)
females from all mothers	744 (59.0)	440 (62.8)	587 (68.7)
males from all mothers	516 (41.0)	261 (37.2)	267 (31.3)

Fig. 1

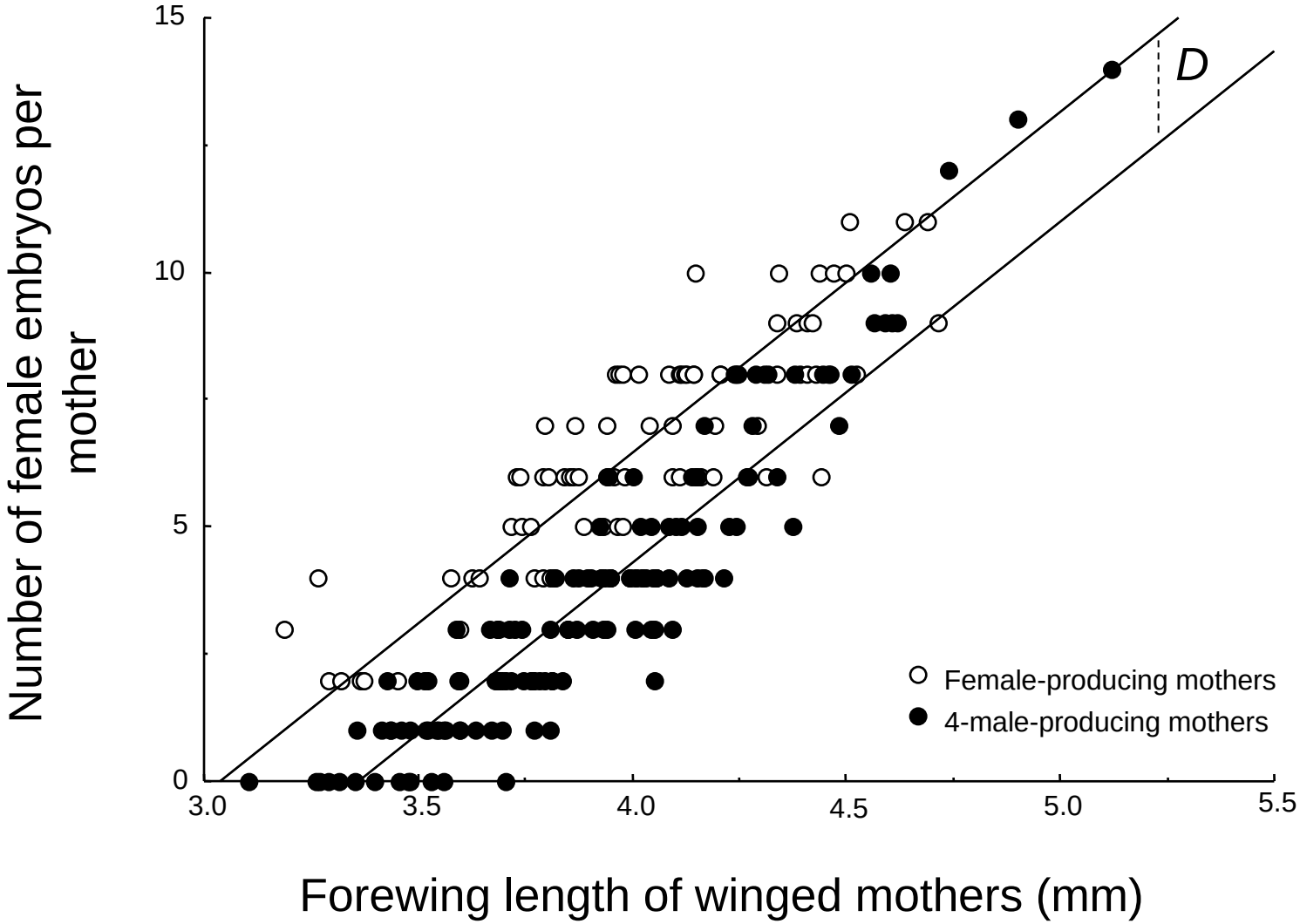
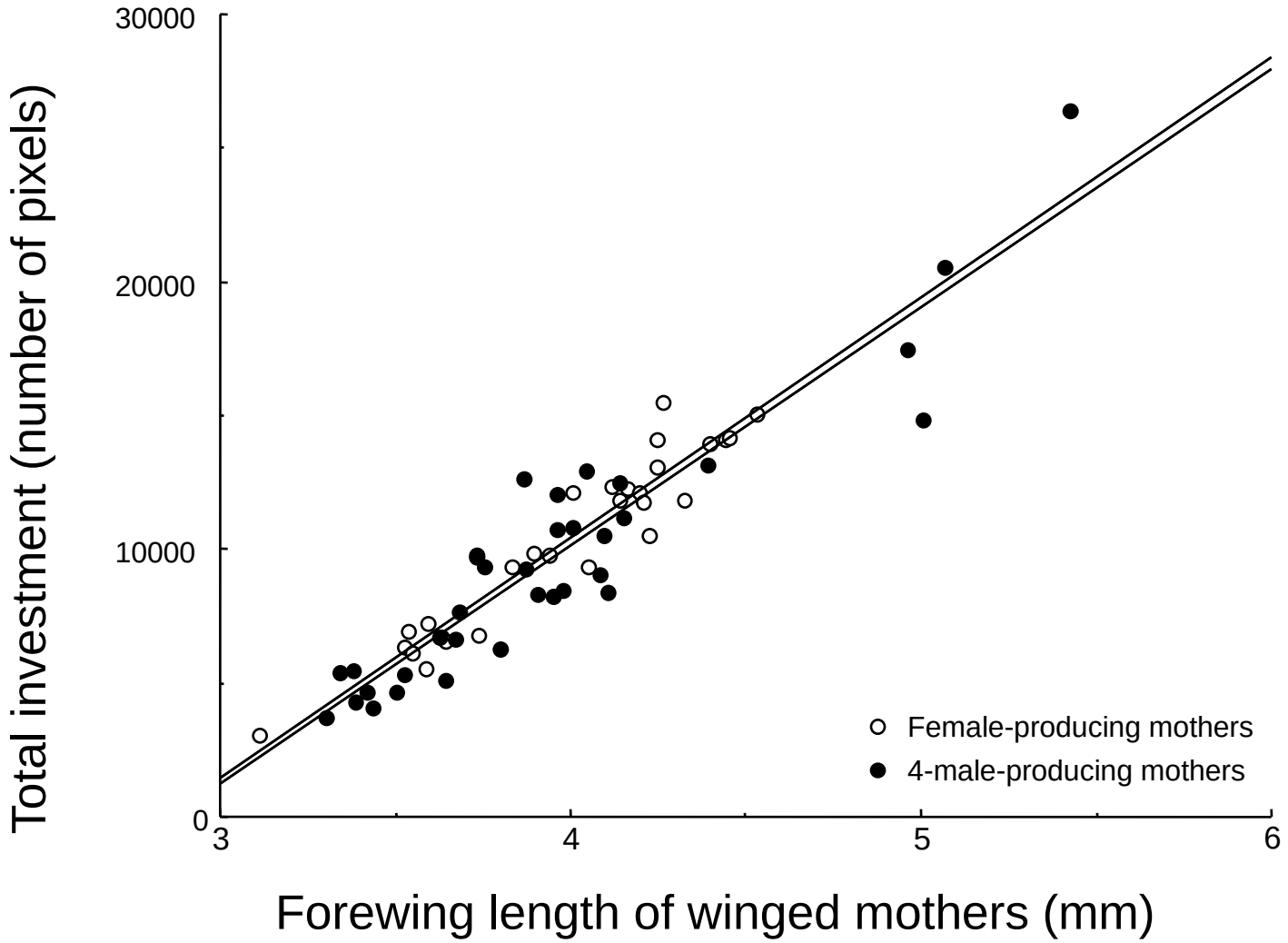
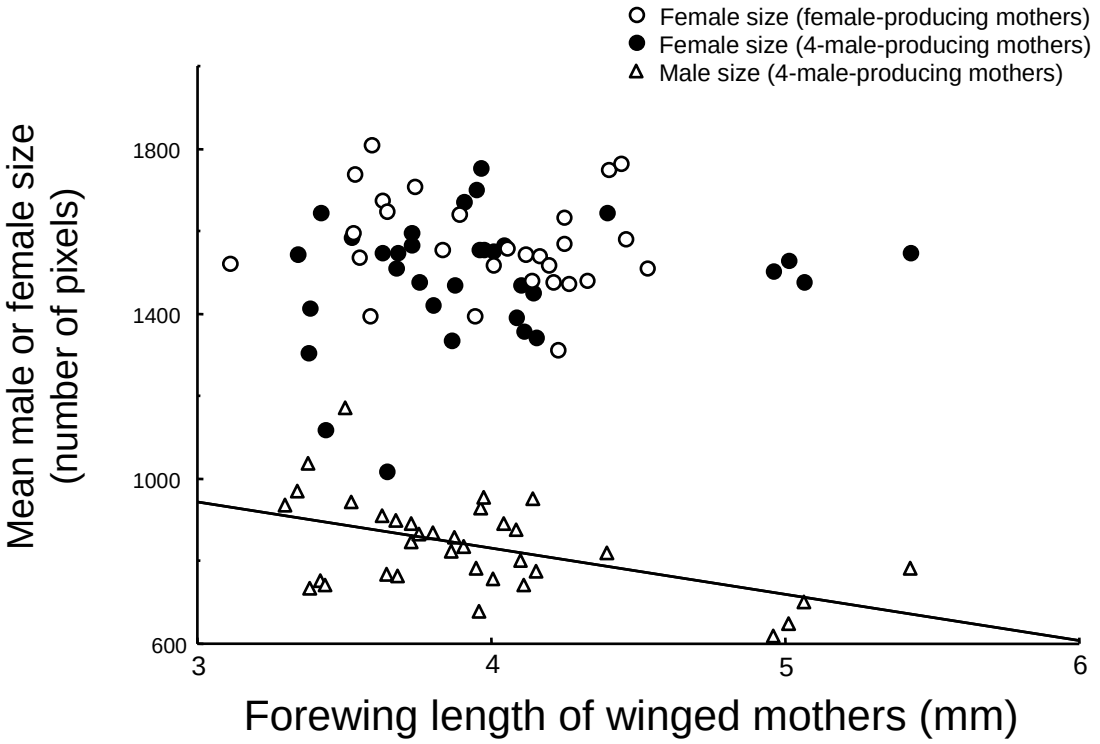

Number of female embryos per mother
15
10
5
0
3.0
3.5
4.0
4.5
5.0
5.5
D
Female-producing mothers
4-male-producing mothers
Forewing length of winged mothers (mm)

Fig. 2


Total investment (number of pixels)
30000
20000
10000
0
3
4
5
6
Forewing length of winged mothers (mm)
Female-producing mothers
4-male-producing mothers


Female size (female-producing mothers)
Female size (4-male-producing mothers)
Male size (4-male-producing mothers)
Mean male or female size (number of pixels)
1800
1400
1000
600
3
4
5
6
Forewing length of winged mothers (mm)

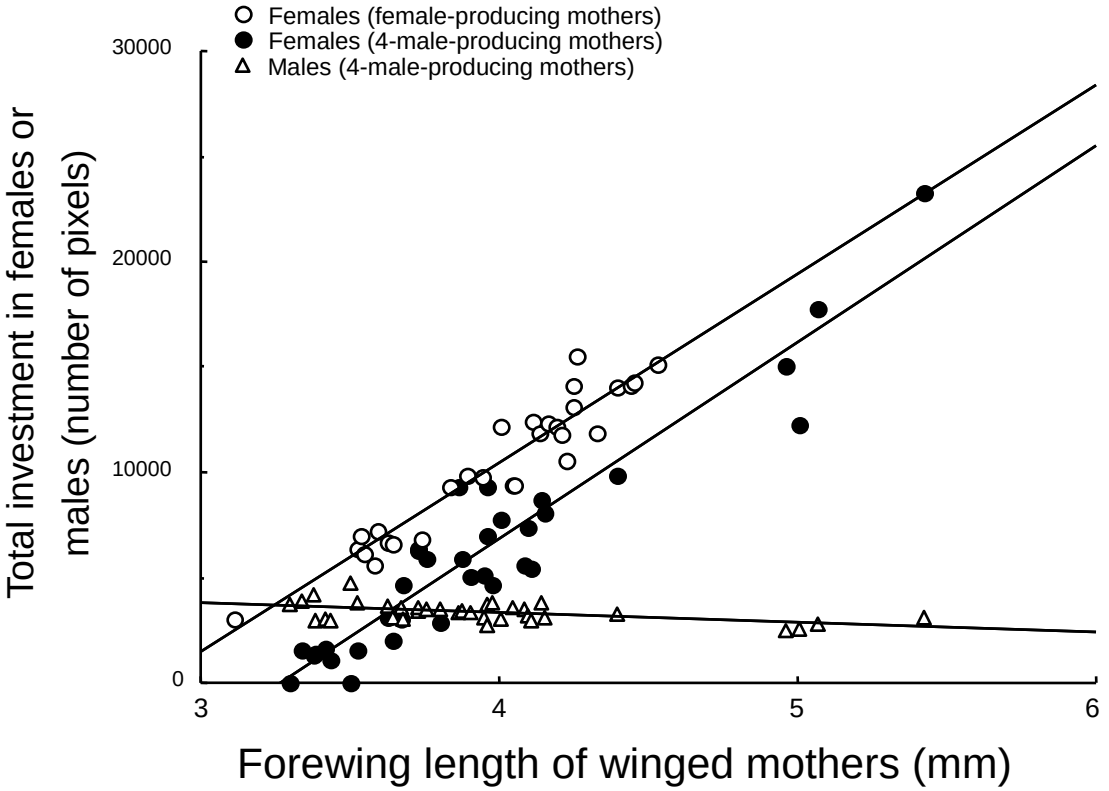
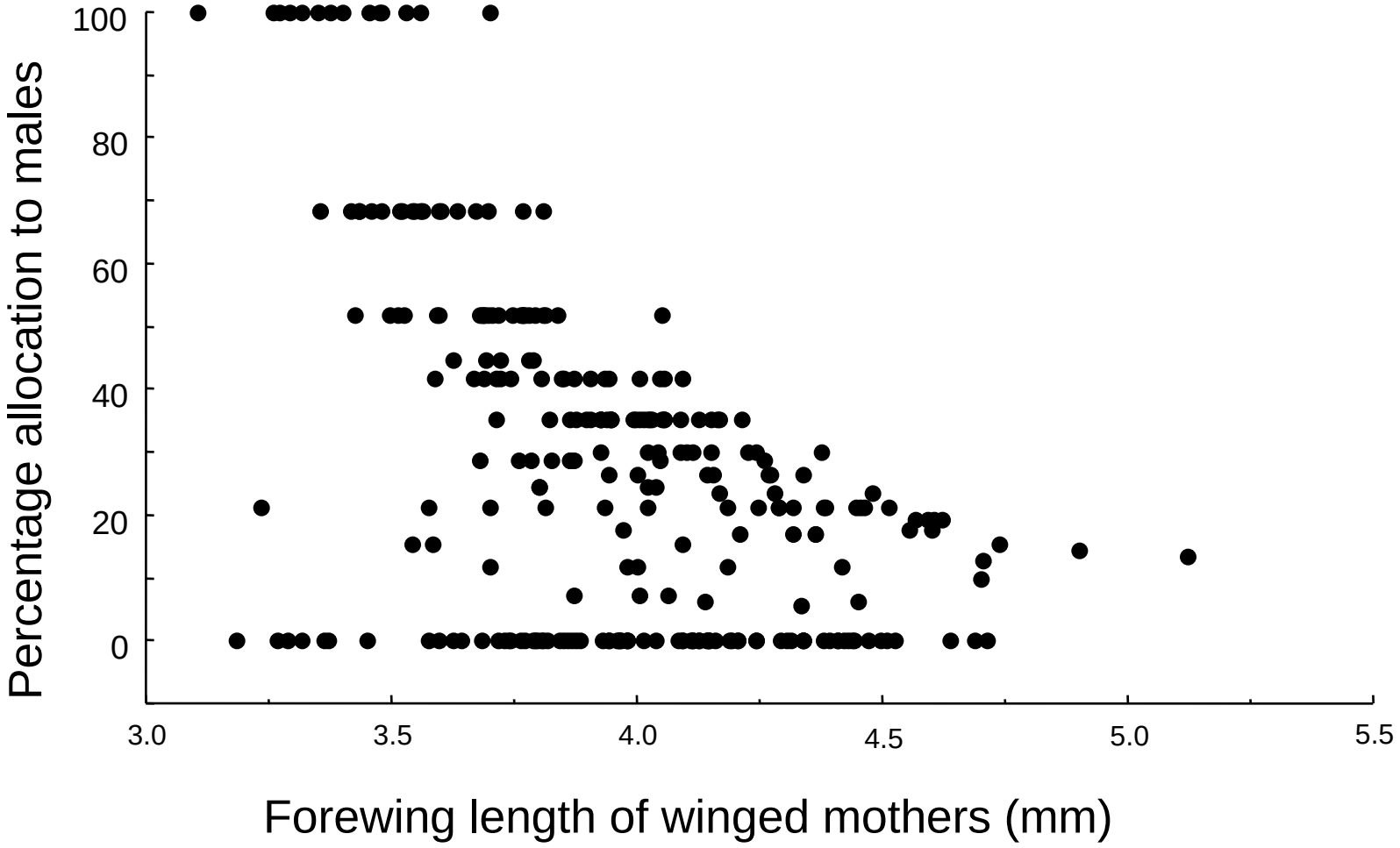

Females (female-producing mothers)
Females (4-male-producing mothers)
Males (4-male-producing mothers)
Total investment in females or males (number of pixels)
30000
20000
10000
0
3
4
5
6
Forewing length of winged mothers (mm)

Fig. 4


Percentage allocation to males
100
80
60
40
20
0
3.0
3.5
4.0
4.5
5.0
5.5
Forewing length of winged mothers (mm)