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Sex ratio and brood size in a monophagous outcrossing gall aphid, *Tamalia coweni* (Homoptera: Aphididae)

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ABSTRACT

Sex allocation theory has been applied successfully in the case of spatially structured aphid populations, in which local mate competition can account for biased sex ratios. Likewise, a demographic effects model can explain sex ratio bias when maternal investment in sons and daughters is asynchronous, owing to developmental constraints. In one of the first studies to examine patterns of sex allocation in a phytophagous insect in which outbreeding is likely and both sexes are produced concurrently, we measure sex ratio and sex allocation for the gall-forming aphid *Tamalia coweni* (Cockerell). While the sex ratio at a low elevation (800 m) site did not differ significantly from 1 : 1, the sex ratio was slightly but significantly female-biased at a higher elevation (1350 m). At both sites, the variance in sex ratio among broods was significantly greater than binomial, suggesting active manipulation of the sex ratio by aphid foundresses. Ten percent of the broods with more than four individuals contained exclusively males or exclusively females, a percentage that could not have resulted from random variation around an average sex ratio. Among non-unisex broods, the sex ratio became increasingly more female-biased with increasing brood size. Local mate competition and non-adaptive demographic effects on the sex ratio could not account for the overall bias towards females. Apart from the possibility of cytoplasmic or other genetic sex ratio distortion elements, the Trivers-Willard hypothesis of condition-dependent sex allocation may best explain the observed sex ratio patterns in *T. coweni*.

Keywords: brood size, gall aphid, sex allocation, sex ratio, *Tamalia coweni*.

INTRODUCTION

Fisher (1930) argued that the reproductive value of the sexes must be equal, since each sex must supply half the ancestry of all future generations in a species. Because of a mating advantage for the minority sex, any differential production of males or females will tend to be checked by natural selection. Among invertebrates, however, strong deviations from equal investment in the sexes are frequently observed, especially in taxa with a haplodiploid

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mechanism of sex determination (Hamilton, 1967; Karlin and Lessard, 1986; Crespi, 1992; Craig and Mopper, 1993). Optimal parental strategy, which would in theory be favoured by natural selection, offers one approach to understanding such sex allocation patterns. An alternative hypothesis is that the sex ratio is simply a consequence of the particular type of sex determination employed (i.e. the cytological machinery) (Karlin and Lessard, 1986). Because of their labile sex allocation mechanism (see below), aphids, as a diplo-diploid group, constitute a rich and under-explored arena in which to test hypotheses concerning adaptive sex ratios (May and Seger, 1985; Foster and Benton, 1992; Moran, 1993). Here, we describe the presence of individually variable sex allocation patterns and a slight but significant overall bias towards females in natural populations of a monophagous outcrossing gall aphid, *Tamalia coweni*. To the authors' knowledge, this is the first systematic study of investment ratios in an aphid in which both sexes are winged.

Control of sex ratio in aphids

In all aphids with a sexual generation, asexual females are produced by apomictic parthenogenesis for most of the growing season, followed by a switch to the production of sexuals; this life-history pattern is termed 'cyclical parthenogenesis'. The change from asexual to sexual production is induced by increased scotoperiod (length of night), lowered temperature, a decline in nutrition, or a combination of these factors (Sethi and Swenson, 1967; Dixon, 1977). Sex determination itself is autosomal and of the type XX/XO: male determination results from the loss of half of the X-chromatin at maturation division of the parthenogenetic egg (Orlando, 1974; Hales and Blackman, 1987). In the aphid *Myzus persicae*, male production is mediated by levels of juvenile hormone (Hales and Mittler, 1983, 1987). Control operates at the level of germarium, the distal section of each ovariole. In some species, a distinct and irreversible switch from female to male production occurs (Forrest, 1970; Blackman, 1980). In others, males are produced in a discrete burst, preceded and followed by female offspring (Orlando, 1974; Hales and Mittler, 1987); in this case there is no irreversible switch by the mother to male production.

Although the sex ratio tends towards 1 : 1 in most organisms with autosomal genetic sex determination, in aphids the sex ratio tends to be female-biased (Dixon, 1985). Two explanations have been proposed for the female bias: an adaptive sex ratio in spatially structured populations and the effect of demography (maternal mortality) on the sex ratio (Karlin and Lessard, 1986). Both scenarios are contingent on the life-history details of the species under consideration.

Adaptive sex ratio in spatially structured aphid populations

A subdivided population structure may be responsible for female-biased sex ratios in, for instance, pemphigine aphids. In these aphids, an entire brood of wingless males and sexual females is deposited by their mother at one place and time. Sib-mating is thus likely and a female bias in maternal investment is expected as a result of local mate competition (Hamilton, 1967). This prediction was confirmed by Yamaguchi (1985), whose model describing investment patterns fit data well for sex ratios in the pemphigine *Prociphilus oriens*. Similarly, Foster and Benton (1992) reported a female bias in the pemphigine *Pemphigus spyrothecae*, where the conditions for local mate competition are met.

Demographic effects on the sex ratio

The timing of maternal mortality may affect the sex ratio in outbred species (Ward and Wellings, 1994a,b). In some aphidine aphids, for instance, males and sexual females are produced separately in time and space and outbreeding is expected. When the life history involves alternation between two host plant species, asexual females on the secondary (herbaceous) host plant produce specialized winged morphs, the gynoparae, which migrate to the primary (woody) host plant before depositing sexual females. The asexual females then switch to production of males, which likewise must migrate to the primary host plant to mate. The additional generation in the life cycle (involving gynoparae) introduces a potential temporal lag or asynchrony between the appearance of sexual females and males. Any increase in early mortality of asexual females (hence fewer surviving to make the reproductive switch from gynoparae to males) will result in a female-biased sex ratio.

This non-adaptive hypothesis was advanced and modelled by Ward and Wellings (1994a,b), who predicted that sex allocation in species undergoing host alternation should range from 0.15 to 0.50 (proportion male). Calculating absolute estimates of sex allocation is difficult in most systems in which sexuals are produced sequentially (Moran, 1993). Nevertheless, field data from studies on the aphidine *Rhopalosiphon padi* confirm that most populations of sexuals are strongly female-biased, although the predicted investment is sensitive to the parameters of larval development time and mortality of asexual females (Ward and Wellings, 1994b). Similarly, Hales *et al.* (1989) found that, under experimentally manipulated conditions of photoperiod on *Myzus persicae*, sex ratio is dependent on maternal survival time.

Biology of *Tamalia coweni*

In *Tamalia* and in a few other non-host-alternating genera of aphidines – *Phloeomyzus*, *Greenidea*, *Aiceona* and *Neophyllaphis* – both sexual females and males are alate (winged), an unusual condition in aphids (Blackman and Eastop, 1994). In the study populations of *T. coweni* in California, two plant species are hosts, *Arctostaphylos viscida* and *Arctostaphylos patula* (Ericaceae). In both species, a wingless stem mother hatches in the spring from the overwintering, sexually produced egg. She induces a gall on a leaf, in which she bears living offspring parthenogenetically; these feed and develop within the gall. The probing activity of the stem mother is the prime determinant of gall size (D.G. Miller, unpublished data); in this sense, the stem mother indirectly provides maternal care for her offspring. On *A. viscida*, offspring are mostly winged males and winged sexual females, which undergo four developmental stages (instars) within the gall before emerging from the gall to complete their fourth and final moult. These sexuals then disperse before mating, completing the life cycle. On *A. patula*, offspring of the stem mothers are mostly winged asexual females, which disperse and deposit another generation of wingless asexual gall-makers (third-generation foundresses), typically on flower buds of *A. patula*. In the flower bud galls, the foundresses then produce winged males and winged sexual females, which disperse on emergence (Miller, 1998).

Because *T. coweni* sexual females disperse before mating, outbreeding is nearly certain; thus, local mate competition is highly unlikely and Fisherian sex allocation may be expected. Contrary to this expectation, we find an overall sex ratio bias in *T. coweni*, which

we try to explain by examining the assumptions of the demographic effects model. We suggest that a third hypothesis not commonly associated with aphid sex allocation patterns – the Trivers-Willard (1973) condition-dependent sex allocation hypothesis – may apply to *T. coweni*. Active manipulation of the sex ratio by *T. coweni* females is suggested by the presence of greater than binomial variance in the sex ratios produced, the presence of unisex broods and a significant increase in the proportion of females with increasing brood size.

METHODS

Field studies

We studied populations of *T. coweni* on *A. viscida* and *A. patula* at Blodgett Forest Research Station, El Dorado County, California (1350 m above sea level), and on *A. viscida* at Georgetown, El Dorado County, California (800 m above sea level). We collected data at the higher elevation during 1994 (Dee Dee Road, DDR) and 1995 (Dee Dee Road and Chinquapin Flat, CF, subsites separated by about 500 m) and at the lower elevation during 1995 only. Each year data collection encompassed the entire period of offspring emergence (July–October). In each collecting season, we selected haphazardly samples of 50 galls from leaves of each host plant species and, during 1994 only, from both leaves and flowers of *A. patula* (Table 1). We placed clip cages over these developing galls before any offspring had emerged (indicated by the absence of cast exoskeletons outside the gall). We monitored the galls until the final offspring emerged, as indicated by drying and shrivelling of the gall, a state no longer conducive to aphid development.

We made a single collection at the end of the season for all samples in 1994. During 1995, we counted all newly emerged adult males and females in the cages once every other day on *A. viscida* at Georgetown (here *T. coweni* completes its life cycle earlier and more quickly than at the higher elevation site) and two to three times per month at Blodgett Forest. After the period of aphid emergence, we collected all sample galls and dissected out and recorded their contents.

We sexed 89% (4403/4939) of offspring in 239 galls sampled, either as adults (3966 emerged into the clip cages) or as juveniles (437 still in the galls when collected). We distinguished live juvenile males easily from females by their pale green colour and conspicuous genital armature (females are dark green and bear inconspicuous genitalia). Dead juveniles or individuals that were otherwise difficult to classify were not sexed but were included in the brood size estimates. In the collected galls, we also recorded the number of adult aphid foundresses.

To determine pre-emergence sex ratios and relative birth order of males and sexual females, we examined the contents of 101 uncaged galls collected from *A. viscida* or *A. patula* before their maturation at the Blodgett Forest site (collections in August and October 1993, July and August 1994, August 1997). For estimates of body length, we measured live or freshly killed aphids with a graticule to the nearest 0.1 mm, excluding antennae and legs. Biomass was estimated to the nearest 0.1 μg on a Cahn C-31 Electrobalance after drying the aphids in an oven at 35°C for at least 24 h.

Statistical analyses

We used two methods to determine whether samples from different host plants, sites and years could be combined in the analyses: nominal logistic analyses (i.e. *G*-tests) of the pooled sex ratios and analysis of variance of the arcsine-transformed proportions of males per brood (0.001 added to the proportions before transformation to eliminate zeroes). These two methods produced similar results when data points in the analyses of variance were weighted by the number of individuals sexed per gall and unisexual galls, which yielded a heavily non-normal distribution, were excluded. We report the results of the nominal logistic analyses only. We used weighted least-squares regression to explore the effect of brood size on the arcsine-transformed proportions. We used least-squares regression rather than logistic regression in this analysis because whole broods were the units most likely to be affected by the covariate brood size and members of the same brood were assumed not to be independent of one another. In the analysis of variance and the least-squares regression, we weighted the data points by the number of individuals sexed per brood, as we considered larger samples to provide more accurate estimates of the actual brood sex ratio.

We applied a Monte Carlo test (Avilés *et al.*, 1999) to determine whether the fraction of unisex broods in the samples was greater than that predicted by chance. We produced 1000 replicate pseudo-datasets by generating broods with an initial size identical to the number of offspring in the sampled broods. From these simulated broods, we then sampled without replacement the number of individuals per brood actually sexed. The sex of the individuals was assigned stochastically when the broods were first produced. Males were produced with a probability equal to the average sex ratio. We compared the observed number and size of all-male and all-female broods in the samples with the distribution of these numbers in the simulated pseudo-datasets.

RESULTS

Population sex ratios and sex allocation

Pooled sex ratios, expressed as the percentage of males among sexual offspring (adults and juveniles), varied from 39.5 to 48.4% in the samples collected (Table 1). These estimates exclude 11 galls that contained asexual females (in addition to sexual females and males), as these had a significantly smaller percentage of males among their sexual offspring (24.5 *vs* 43.1% males, pooled across galls; $G = 23.5$, d.f. = 1, $P < 0.0001$). We found no significant differences in the sex ratios across subsites (DDR *vs* CF) or the two plant types (*A. patula vs A. viscida*) at the higher elevation site (Table 1) (subsite effect: Wald $\chi^2 = 0.64$, d.f. = 1, $P = 0.43$; plant effect: Wald $\chi^2 = 0.20$, d.f. = 1, $P = 0.66$). We therefore combine these samples for further analyses. As we detected significant differences in the sex ratio across years at the higher elevation site (Wald $\chi^2 = 4.79$, d.f. = 1, $P = 0.03$) and between the two elevations in 1995 ($G = 17.0$, d.f. = 1, $P < 0.0001$), we consider these samples separately in the analyses, treating them as blocks when appropriate.

The overall proportion of males was slightly but significantly female-biased (43.1% males, $G = 81.8$, d.f. = 1, $P < 0.0001$, $n = 228$ galls lacking asexual offspring). There were, however, differences among blocks. The sex ratio at the higher elevation departed significantly from 1 : 1 in 1994 (44.3% males, $G = 14.0$, d.f. = 1, $P = 0.0002$) and 1995 (40.4% males, $G = 84.7$, d.f. = 1, $P < 0.0001$). In contrast, the sex ratio at the lower elevation,

Table 1. Numerical sex ratios of *Tamalia coweni* offspring (sexual adults plus juveniles, excluding 11 galls that contained asexuals)

Location	Elevation	Year	Gall type	No. of galls	Brood size[a]	Percent males[b]	Total males	Total females	Percent males (pooled)
Blodgett Forest, DDR	High	1994	*A. patula* leaf, flower galls	40	16.6 ± 2.2	47.5 ± 5.3	243	324	42.9
Blodgett Forest, DDR	High	1994	*A. viscida* leaf galls	37	15.2 ± 1.7	40.1 ± 4.9	227	268	45.9
Blodgett Forest, DDR	High	1995	*A. patula* leaf galls	29	28.0 ± 2.0	44.8 ± 3.5	297	432	40.7
Blodgett Forest, DDR	High	1995	*A. viscida* leaf galls	47	23.4 ± 1.8	37.3 ± 3.2	407	623	39.5
Blodgett Forest, CF	High	1995	*A. viscida* leaf galls	31	17.7 ± 2.0	36.2 ± 4.4	243	303	41.6
Georgetown	Low	1995	*A. viscida* leaf galls	44	22.9 ± 1.9	48.3 ± 3.8	439	464	48.4
All samples				228	20.6 ± 0.8	43.0 ± 1.8	1826	2414	43.1

[a]Brood size estimated from entire contents of the galls, including both sexually differentiated and undifferentiated individuals (mean ± standard error).
[b]Percentage of males estimated among adult and juvenile sexuals in a gall (mean ± standard error).

estimated during 1995 only, did not differ significantly from 1 : 1 (48.4% males, $G = 0.87$, d.f. = 1, $P = 0.35$).

There were no significant differences in mean (± standard error) body length between first-instar sexual females (0.74 ± 0.03 mm, $n = 39$) and males (0.72 ± 0.03 mm, $n = 29$) (t-test, $t = 0.549$, $P = 0.585$). However, adult females were heavier than adult males. The dry mass of adult aphids ranged from 0.018 to 0.047 mg in males (0.034 ± 0.001 mg) and from 0.023 to 0.111 mg in females (0.072 ± 0.003 mg); the means differed significantly ($n = 50$ each, $t = 11.836$, $P < 0.001$). Therefore, if the period of development within the galls can be considered part of the period of parental investment, investment ratios would be even more biased than the numerical ratios reported.

To determine whether differential mortality could account for the observed sex ratio bias, numerical ratios for first-instar and fourth-instar juveniles were estimated. In the 101 uncaged galls collected separately and dissected before maturity, sex ratios were already significantly female-biased in both age classes: pooled sex ratio = 0.34 in first-instar individuals ($n = 73$ aphids, $\chi^2 = 7.247$, $P = 0.001$) and 0.29 in fourth-instar individuals ($n = 458$, $\chi^2 = 80.489$, $P < 0.0001$).

Developmental rates of males and sexual females

We found no support for the demographic effects model (Ward and Wellings, 1994a,b) as an explanation for the overall sex ratio bias in *T. coweni*. The emergence periods of males and females overlapped broadly or were more brief for males than for females. Twenty-three of the 101 galls collected at the higher elevation site before maturity contained juveniles of both sexes, including at least one first-instar individual of either sex. In 15 of these, all first instars were sexual females, in three they were all males and in five first instars of both sexes were present. At this site in 1994, males apparently completed development somewhat earlier than sexual females: 78% of males ($n = 60$ individuals) and 74% of sexual females ($n = 96$ individuals) had already emerged from galls collected on *A. viscida* by 10 August. In galls collected between 18 and 24 August, 100% of males ($n = 23$ individuals) had completed development, whereas 35% of sexual females were still in their fourth instar ($n = 34$) (the apparent inconsistency in developmental rate of sexual females probably reflects sampling error). In galls on *A. patula* flower buds, 88% of males ($n = 69$) and only 66% of sexual females ($n = 140$) had emerged by 24 August; this late in the season, remaining juvenile sexual females were unlikely to complete development before resource quality had diminished greatly.

For the 50 *A. viscida* galls monitored at the lower elevation site in 1995, the emergence of sexual females and males overlapped broadly, although sexual females outnumbered males both at the beginning and end of the eclosion period (Fig. 1). Emergence periods ranged from the 2.84 ± 0.52 days for males to 5.48 ± 1.05 days for females. In each gall, the first appearance of sexual females preceded that of males by 0.29 ± 0.21 days; last appearance of sexual females succeeded that of males by 2.03 ± 0.89 days. During 1995, the entire period of emergence of sexuals in this population was from early July to mid-August.

Inter-gall sex ratio variation

Gall sex ratios ranged from 0 to 100% female, with significant heterogeneity among galls in the three blocks ($G = 294.5$, d.f. = 76, $P < 0.0001$, for the high elevation site in 1994;

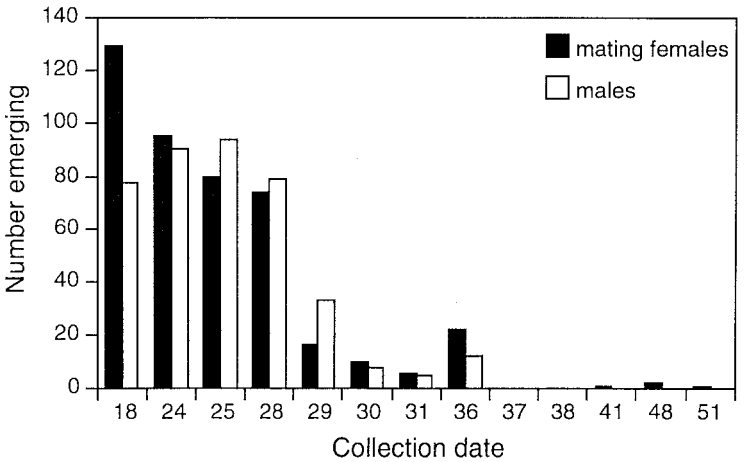

Fig. 1. The appearance of adult sexuals in a population of *Tamalia coweni* on *Arctostaphylos viscida* (total sexual females = 433; males = 399). Data from 50 galls monitored on 17 days between 20 June and 10 August 1995 at Georgetown, California. Emergents were collected from cages placed over galls; collection date = number of days from beginning of study.

$G = 317.6$, d.f. = 106, $P < 0.0001$, for the high elevation site in 1995; $G = 124.6$, d.f. = 43, $P < 0.0001$, for the low elevation site in 1995). We identified two potential sources for this overdispersion: an unusually large number of unisex broods and an inverse relationship between brood size and the proportion of males in a brood.

Unisex broods

Forty-eight of the 228 galls lacking asexuals and four of the 11 galls with asexuals contained exclusively members of one sex among the sexual adults and juveniles sexed. If we exclude those broods with fewer than five sexed individuals (which have a $P > 0.05$ of being unisex by chance alone), 24 broods contained exclusively females (range 5–31 females) and five exclusively males (range 5–11 males). A Monte Carlo test shows that, given observed brood sizes, the number of individuals sexed and the overall sex ratio in the original sample, the production of 13 all-male and 39 all-female broods by chance alone is statistically extremely improbable ($P = 0.004$ for the production of 13 or more all-male broods and $P < 0.00001$ for the production or 39 or more all-female broods) (Figs 2a,c). Furthermore, unisexual broods in the simulations contained at the most 9 males or 13 females, while those in the actual samples contained up to 11 males and 31 females (Figs 2b,d). These results show that unisex broods represent an alternative sex allocation pattern that females with small- to medium-sized broods may exhibit, whether resulting from adaptive or non-adaptive causes (see Discussion).

In our samples, unisex broods were significantly more likely among small- and medium-sized broods (Wald $\chi^2 = 40.5$, d.f. = 3, $P < 0.001$, logistic regression with brood size nested within block; block effect non-significant). Also, all-female broods were significantly more common ($G = 13.6$, d.f. = 1, $P = 0.0002$, 52 unisex galls with or without asexuals) and larger ($t = 1.664$, d.f. = 50, one-sided $P = 0.05$) than all-male broods.

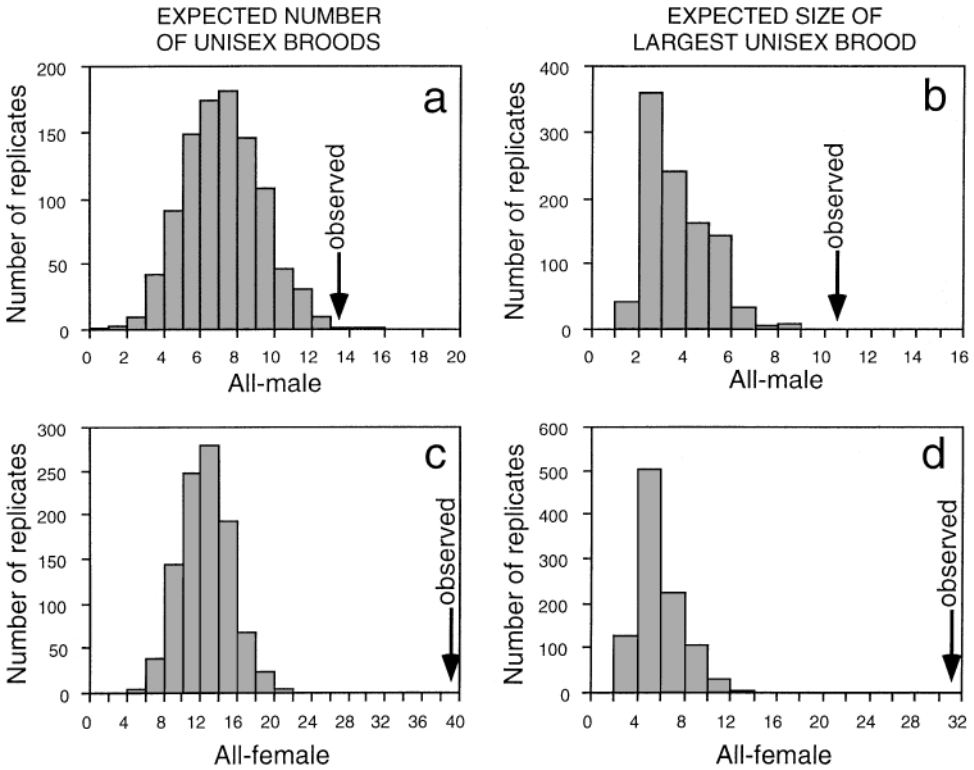

Fig. 2. Monte Carlo simulation results showing distribution of expected number and expected maximum size of all-male and all-female broods based on the null hypothesis that the number of males per brood is binomially distributed with probability equal to the overall population sex ratio. Distributions show results of 1000 pseudo-datasets. Observed values are at the extreme (13 all-male broods; $P = 0.0004$ of the replicates with 13 or more all-male broods; Fig. 2a) or beyond the extreme end of the distribution (11 males in the largest all-male brood, 39 all-female broods and 31 females in the largest all-female brood; Figs 2b, 2c and 2d, respectively). These results allow us to strongly reject the null hypothesis.

Effect of brood size on the sex ratio

To determine the effect of brood size on sex ratio, we treated both elevations and the two years at the higher elevation site as blocks, with brood size nested within blocks. We used arcsine-transformed proportions of males as our dependent variable, weighting each data point by the number of individuals sexed in a brood. We excluded from the analysis broods with five or fewer offspring sexed, as we considered estimates of sex ratio in very small samples to be disproportionately affected by sampling error. We also excluded from these analyses all unisex broods because we believed these represented an alternative sex allocation strategy, as discussed in the previous section. Galls with asexuals were also excluded.

We found that brood size had a significant effect on the sex ratio ($F = 14.6$, d.f. = 3, $P < 0.0001$), with larger broods containing a smaller proportion of males. This effect was highly significant within two of the blocks and marginally non-significant in the third block

($t = -3.7$, $P = 0.0003$, for the high elevation site in 1994; $t = -5.2$, $P < 0.0001$, for the high elevation site in 1995; $t = -1.9$, $P = 0.06$, for the low elevation site in 1995). Neither the slopes of the regressions nor the intercepts were significantly different from each other across blocks ($F = 0.52$, d.f. = 2, $P = 0.6$, for the block effect; $F = 1.31$, d.f. = 2, $P = 0.3$, for the interaction between block and brood size in an analysis of covariance). We thus combined the three blocks in a single weighted least-squares regression analysis, as shown in Fig. 3.

It would appear that the differences in mean sex ratio between years at the high elevation site (see 'Population sex ratios and sex allocation') may have resulted from differences in mean brood size across years. We found that the mean brood size at the higher elevation was larger during 1995 (excluding galls with asexuals, 23.0 ± 1.2) than 1994 (15.9 ± 1.4), a result that was statistically significant ($F_{1,182} = 15.4$, $P < 0.0001$). This brood size difference was possibly an indirect consequence of the greater annual precipitation in 1995, which was three times that of 1994 (262 *vs* 87 cm). On the other hand, there were no significant differences in mean brood size between the high and low elevation sites during 1995 ($F_{1,149} = 0.003$, $P = 0.96$). The difference in mean sex ratio at the two elevations, therefore, remains unclear.

DISCUSSION

Labile sex allocation patterns in *T. coweni* are complex but may represent an adaptive response to variation in the environment, as elaborated below. Overall population sex ratios were skewed significantly towards females; brood sex ratios in large families were especially female-biased. The observed sex ratios were not the result of differential mortality of

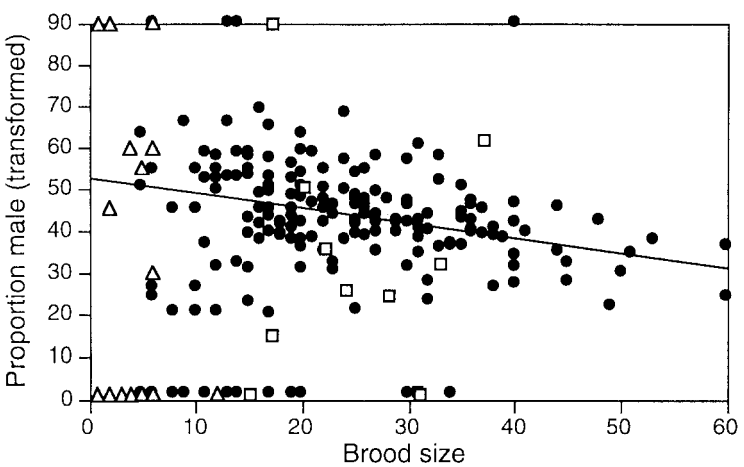

Fig. 3. Proportion of *Tamalia coweni* offspring per gall (arcsine transformed, in degrees) plotted against brood size ($n = 239$ galls). Among non-unisex galls, *T. coweni* foundresses invest in a continuously decreasing proportion of male offspring as brood size increases. The solid trend line denotes a weighted least-squares regression of sex ratio on brood size ($y = -0.353x + 52.47$, $P < 0.0001$; $R^2 = 0.19$), excluding broods with fewer than five individuals sexed (△), broods containing asexuals (□) and unisex broods. The large all-male data point at the top of the graph corresponds to a brood with only five individuals sexed.

juvenile sexuals or of adult foundresses and, therefore, may be due to maternal investment decisions.

Skews in population- and brood-level sex allocation

Although brood sex ratio varied greatly among the populations sampled, we found an overall female bias that is inconsistent with expected Fisherian investment patterns. Because male and sexual female body length at the first instar is nearly equivalent, the numerical sex ratio at this stage approximates sex allocation in *T. coweni*, in agreement with data for the aphid *Myzus persicae* (Hales *et al.*, 1989). However, if parental expenditure is considered to include development of offspring within the gall, overall female bias is magnified, as adult female sexuals were significantly larger than males. We found no evidence that differential mortality can account for the observed female bias in population sex ratios.

Yamaguchi (1985) first demonstrated precise sex ratios (i.e. brood sex ratio variance significantly lower than binomial expectation) in the aphid *Prociphilus oriens*, and both greater than binomial and precise sex ratios have been well documented in parasitoid Hymenoptera (Hardy, 1992). In *T. coweni*, there was significant heterogeneity in brood sex ratios (i.e. greater than binomial variance), apparently the result of an inordinate production of all-male or all-female broods and of an increase in the proportion of females with increasing brood size. These two findings raise the possibility of active manipulation of the sex ratio (Hardy *et al.*, 1992) in this aphid species.

Demographic effects on the sex ratio

Although the actual sequence of male and female embryo production in *T. coweni* is unknown, the results of this study do not suggest an irreversible switch from production of females to males. Adult males are significantly smaller than adult sexual females and apparently complete development more quickly, although the appearance of adult females typically both precedes and succeeds that of males in galls with both sexes present. Thus, early-emerging sexual females are most likely to be the first offspring born in a given brood. Because late-emerging females generally appear after the latest males, female-biased sex ratios in *T. coweni* are not an artifact of incomplete development of males (Fig. 1). Consequently, the demographic effects model is insufficient to account for skewed sex allocation in *T. coweni*.

In the sense of the aphid clone as an evolutionary individual (Janzen, 1977), *T. coweni* clones may be considered simultaneously hermaphroditic (i.e. they invest in both sperm and ova at the same time). The maturation of the gall itself may well play a role in enforcing synchrony in emergence of adult sexuals in *T. coweni*. Fourth-instar males and females may be forced to delay completion of development until the mature gall dries and splits along a suture, allowing release of its occupants (Forrest, 1987).

Condition-dependent sex allocation

As already mentioned, local mate competition is an unlikely explanation for the overall sex ratio bias in a species where both sexes are winged and outbreeding is expected. Therefore, we would like to suggest a third hypothesis, not commonly associated with aphid sex ratios, as a potential explanation for the sex allocation patterns of *T. coweni*. Trivers and Willard

(1973) developed a hypothesis predicting maternal investment patterns that would reflect the parental ability to invest in one sex or the other. Although their argument makes specific reference to a male bias in those species in which male reproductive success exhibits greater variance than that of female reproductive success, it can be generalized to the following: females in good condition are expected to invest in the sex for which quality makes the greatest difference in reproductive success (Clutton-Brock *et al.*, 1984). In years when environmental conditions are uniformly better or worse than average for most members of a population, then population-wide sex ratio biases may result.

A facultative sex allocation response to variable environmental conditions (e.g. host size and quality) is well-documented and observed in mermithid nematodes and parasitoid wasps (Clausen, 1939; Charnov, 1982; Fox *et al.*, 1996), and may apply to sex allocation in *T. coweni*. In flowering plants, stress apparently biases sex allocation towards the male function (Karlin and Lessard, 1986); similarly, in the gall-making sawfly *Euura lasiolepis*, population and family sex ratios were more female-biased in host plants undergoing accelerated growth under both natural and experimental conditions (Craig *et al.*, 1992). These shifts in sex allocation may be based on size-dependent relative fitness gains of sons and daughters (male and female function). In many insects, large size, which may be a result of favourable environmental conditions, is more closely correlated with female fitness than with male fitness (Van den Assem *et al.*, 1989; Hardy *et al.*, 1992; Ueno, 1998; but see Clutton-Brock *et al.*, 1984, 1997; Crespi, 1986, 1988); consequently, under favourable conditions, sex allocation should be skewed towards females. That fecundity is a correlate of body size in *T. coweni* is a reasonable assumption: in the aphids *Masonaphis maxima*, *Sitobion avenae* and *Pemphigus spyrothecae*, larger females are both more fecund and bias sex allocation towards daughters rather than sons (Gilbert, 1980; Newton and Dixon, 1987; Foster and Benton, 1992); additionally, size and fecundity in aphids are generally positively related with nutrition (Moran, 1993).

Greater investment in males under deteriorating conditions is also seen in the aphids *Masonaphis maxima* and *Uroleucon gravicorne* and the sawflies *Neodiprion edulicolis* and *Euura lasiolepis*, where sex ratio is inversely related to host plant quality, across hosts of the same or different species (Gilbert, 1980; Craig *et al.*, 1992; Mopper and Whitham, 1992; Moran, 1993). A related hypothesis for adjustment of sex ratio under stress was proposed by Myers (1978), who argued that poor nutritional conditions so influence investment decisions that maximization of the number of the least 'expensive' sex is the outcome. Because male and female *T. coweni* are apparently equally costly at birth, however, this hypothesis cannot be tested with the present dataset.

Unisex broods

Although condition-dependent sex ratio allocation may help explain female bias in large broods and in the early-season galls, a considerable number of small all-female broods occurred in this study, indicating that foundresses are most likely to invest in females when expected productivity is smaller than average, as well as when it is larger than average (Fig. 3). Alternatively, these unisex broods may be due to failure of the maternal cytological mechanism necessary to produce males, or they may be the manifestation of a developmental cost associated with switching to production of males. Such a cost has been found in at least two aphid species: abnormal follicles, which are likely to result in abortive larvae, occur frequently in the mother's ovariole during the transition from female to male pro-

duction in *Megoura viciae* and *Myzus persicae* (Orlando, 1974; Searle and Mittler, 1981). Finally, microbially induced sex ratio distorters (Taylor, 1990; Wilkinson, 1998) could account for the overall female bias in *T. coweni*, although genetic screening for *Wolbachia*, a bacterium known to induce female bias in some insects, proved negative (D. Shoemaker, personal communication).

CONCLUSIONS

Local mate competition and non-adaptive demographic effects on the sex ratio appear to be unlikely explanations for the sex allocation patterns of *T. coweni*. The results presented here may best fit the Trivers-Willard hypothesis. However, rigorous experimental work on the factors of host plant nutrition, maternal fecundity and the mechanisms of sex determination in *T. coweni* is needed before firm conclusions can be drawn regarding the adaptive nature of these patterns. Sex ratio allocation patterns in *T. coweni*, and indeed in aphids generally, are probably the outcome of complex interactions of life-history parameters, developmental mechanisms and environmental effects acting at the level of the individual and the aphid clone. Aphid sex ratios do not generally conform to Fisherian expectation and may be only partially tractable to optimality criteria. Therefore, it is important to determine the critical assumptions underlying theories of adaptive sex ratio (Frank, 1990). Indeed, using the approach of evolutionarily stable strategies modelling, Bull (1981) and Frank (1987) showed population sex ratios of zygotes may not be 0.5 at equilibrium when there are non-linear returns on investment: the predicted sex ratio depends on the shape and location of the relative fitness curves for male and female function and is correlated with an environmental parameter such as nutritional quality. Hales *et al*. (1989) rightly conclude that it is too early to make general statements about sex allocation in aphids.

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