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Evidence for adaptive sex allocation in *Tamalia coweni* (Hemiptera: Aphididae) in response to nutrient variability in *Arctostaphylos patula*

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ABSTRACT

Question: Do animal species with facultative sex allocation invest adaptively according to local environmental conditions?

Hypothesis: The Trivers-Willard hypothesis can explain maternal sex allocation patterns in response to experimental manipulations in the field.

Organisms: A galling aphid, *Tamalia coweni*, and its host plant, *Arctostaphylos patula*.

Field site: Lassen National Forest, Butte County, California, USA, 1700 m above sea level.

Methods: Using a randomized block design we enriched host plants with various concentrations of nitrogen (urea), then recorded sex ratios and mass of developing male and female offspring, as well as female fecundity.

Conclusions: Aphid foundresses respond to nitrogen enrichment by skewing brood sex ratios towards daughters. We observed no changes in *T. coweni* body mass or its number of presumptive ova.

Keywords: aphid, gall, nitrogen, parental investment, sex ratio, Trivers-Willard hypothesis.

INTRODUCTION

Fisher (1930) used straightforward logic to explain why sex ratios tend to be equal in many species, a conundrum bedevilling Darwin some 60 years earlier (Darwin, 1871). Fisher gained his insights by considering sex ratio patterns across multiple generations, suggesting that negative frequency-dependent selection can favour parents investing in the sex in shortest supply. However, biased population-wide sex ratios have been characterized both empirically and theoretically, especially among invertebrates (Craig and Mopper, 1993; West, 2009). More precisely, patterns of parental investment in sons and daughters (i.e. sex allocation) are frequently skewed away from equilibrium, owing to a multitude of factors including selection. Manipulating sex ratios can be advantageous when differential payoffs on

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investment in males versus females apply (Charnov and Bull, 1989). Competing hypotheses have been advanced to explain sex allocation in a variety of animal species, especially those with labile sex allocation, although direct experimental evidence supporting these hypotheses has been demonstrated only infrequently in field-based studies (Craig *et al.*, 1992).

Sex allocation in aphids may be viewed as an optimization problem free of the potential conflict over investment decisions otherwise inherent between the sexes or within genetically heterogeneous social insect colonies (Ratnieks and Reeve, 1992; West, 2009). A single aphid genotype typically comprises a diverse series of morphs functionally specialized to perform a clonal division of labour. The aphid clone is exquisitely adapted to respond to subtle variations in resource availability in time and space (Dixon, 2005). Although there have been few investigations on sex allocation in aphids, those species studied tend to be female-biased, with up to 20 females for every male in some populations (Dixon, 1998).

Hamilton (1967) realized the importance of population structure in explaining deviations from equal investment in the sexes, arguing that, in viscous populations, local mate competition could skew sex ratios towards females when inbreeding was likely and male siblings competed for mates. Among invertebrates, examples of local mate competition are seen in mites, parasitoid wasps and many aphids, notably those with flightless sexual stages (Yamaguchi, 1985; Aoki *et al.*, 1996; West, 2009). Biased sex ratios can also result from local resource competition among females, favouring broods with an excess of males (Hamilton, 1967; Karlin and Lessard, 1986). Under typical Fisherian sex ratio selection, the primary sex ratio (ratio at birth) is predicted to follow equal investment patterns; subsequently, sex-specific mortality factors may skew sex ratios at adulthood (Charnov and Bull, 1989; Komdeur and Pen, 2002).

When resource quality varies in time and space, natural selection can favour environmental sex determination, as witnessed in temperature-dependent sex determination in reptiles (Langkilde and Shine, 2005). The Trivers-Willard hypothesis, a special case of parentally mediated environmental sex determination, was originally formulated to explain deviations from a 50/50 sex ratio in ungulates (Trivers and Willard, 1973; Charnov, 1982). It has since been successfully generalized to a wide variety of species exhibiting adaptive, conditional sex allocation: the theory predicts that the sex gaining the most fitness in high-quality environments should be produced under favourable conditions, whereas the other sex should be produced under poor conditions (West, 2009). For the Trivers-Willard hypothesis to apply, parental investment must be correlated with the condition of the mother, condition of the young must endure to adulthood, and male parental investment must be negligible (Trivers and Willard, 1973).

Empirical and theoretical tests of the Trivers-Willard hypothesis abound in the literature (West, 2009). For example, parasitic nematodes with labile sex ratios produce female-biased broods under favourable nutritional conditions; worms developing under crowded circumstances with competition over food resources are more likely to become males (Trudgill, 1967). Many insects actively manipulate sex ratios in response to ambient conditions (Waage and Lane, 1984; Ratnieks and Boomsma, 1995). Parents may vary allocation of resources in terms of abundance, quality or duration: all represent forms of differential investment in offspring. A good example of condition-dependent sex ratio manipulation in a field-based system is seen in the sawfly *Euura lasiolepis*, in which there are unequal fitness payoffs from male and female progeny. Craig *et al.* (1992) reported that *E. lasiolepis* exhibits adaptive responses to variation in host plant quality through differential investment in males and females.

Nutritional resources can generate some of the most powerful selective forces shaping life-history traits (Tallamy and Wood, 1986). Nitrogen, particularly in the form of nitrate, is

widely considered the nutrient with the strongest influence on community productivity in terrestrial ecosystems (Townsend *et al.*, 2008). Research on desert communities has revealed positive correlations between increased foliar nitrogen content and phytophagous insect densities (Lightfoot and Whitford, 1987). In many aphid species, growth and reproduction are limited by levels of usable nitrogen, a nutrient only minimally present in their principal diet, plant phloem (Whitham, 1978; Dixon, 1998; Whitehead *et al.*, 1992). Nevertheless, aphids exhibit highly efficient assimilation of phloem nitrogen, incorporating up to 73% of their total nitrogen budget into insect biomass (Mittler, 1958; Van Hook *et al.*, 1980).

Aphids can respond to resource quality and quantity by varying investment patterns in offspring. For example, Brough and Dixon (1989) found an intra-clonal trade-off between reproductive investment in the size of gonads and fat bodies in the vetch aphid (*Megoura viciae*), which highlights the ability of this species to balance periods of nutrient deprivation against opportunities for exploiting high-quality habitats. In other species, higher quality resources yield larger offspring (Whitham, 1978; Nevo and Coll, 2001). Adults of several species display up to a 10-fold range in body size with improving host-plant quality and temperature; large body size is correlated with the number and size of offspring produced (Wellings *et al.*, 1980; Kindlmann *et al.*, 1992; Dixon, 2005). Parental investment in aphids has been quantified in number of offspring produced, ratio of clutch weight to body weight, and biomass of the offspring (Ward *et al.*, 1983). The great flexibility seen in aphid life cycles, involving seasonal alternation between winged and wingless forms, is consistent with tight coupling between investment patterns in the aphid clone and temporal variation in host-plant quality.

In a previous study, Miller and Avilés (2000) showed foundresses in natural populations of the gall-forming aphid *Tamalia coweni* actively manipulate offspring sex ratios in a manner consistent with the Trivers-Willard hypothesis. Here we explore whether gall foundresses respond adaptively to experimental manipulations of the host plant, thus providing a direct test of this hypothesis. We predicted that, following nitrogen enrichment treatments, *T. coweni* foundresses would invest preferentially in female offspring in accordance with the level of enrichment.

MATERIALS AND METHODS

Study system

Tamalia coweni induces galls on manzanita shrubs (Ericaceae) in northern and western North America. Our study population of *T. coweni* and its host plant *Arctostaphylos patula* occurred at Humboldt Summit, Butte County, California, where the Cascade Range and Sierra Nevada meet (1700 m a.s.l.). Here, *A. patula* grows in discrete patches on an average slope of 40%. Mean precipitation exceeds 150 cm per annum, with the majority falling in winter and spring (Western Regional Climate Center, 2012).

As with the majority of aphid life histories, *T. coweni* undergoes a series of parthenogenetic generations culminating with sexual morphs at the end of the growing season (Dixon, 1998), from which the recombinant eggs enter diapause. In spring, a wingless stem mother hatches and induces a gall on a new leaf. Through her feeding activities, the stem mother continues to stimulate gall expansion, after which she bears up to 52 live alatoid (presumptively wing-bearing) young within the gall (Miller, 1998). Each of the five morphs in the life cycle passes through four developmental stages or instars before adulthood. Although offspring are borne serially over the course of 3–4 weeks, most exit the gall over a

comparatively brief 2–3 days. This synchrony of maturation appears to result from the enclosure of the young in the gall until it splits open, releasing its occupants. The stem mother's daughters (winged females) complete their fourth and final moult outside the gall, after which they disperse aerially, each producing 8–17 wingless gall-inducers (foundresses) on still-growing organs of the host plant, typically the inflorescences. Because host plant leaf flush precedes the appearance of inflorescence buds by 6–9 weeks, *T. coweni* can exploit both plant organs sequentially. The foundresses induce another generation of galls and produce the final, sexual stages including winged males and, remarkably, winged sexual females, otherwise rare in aphids (Miller, 1998).

Because the sexes emerge synchronously, the *Tamalia* clone may be regarded as a simultaneous hermaphrodite (Miller and Avilés, 2000). Mating takes place after dispersal from the natal gall, virtually assuring outcrossing. The presence of both winged sexual females and outcrossing distinguishes this life history from that of most other aphid species, in which the sexual females are wingless and inbreeding is more likely: this arrangement tends to reduce the probability of clonal selfing (Dixon, 2005). In an effort to simplify our investigation of optimal sex allocation in *T. coweni*, we limited our analysis to the sexual generation only.

Experimental protocol

Our experimental work and data collection took place in 2005–2006. To compensate for differences in slope, aspect, and host-plant spatial distribution, we used a randomized block experimental design. We delineated eight 15×15 m blocks and randomly tagged 48 plants within these. We selected X and Y coordinates randomly; we used these to identify the location of plants within blocks. Thus we obtained two plants per treatment per block, giving 16 plants per treatment overall.

We standardized the selection of study plants by excluding those less than 0.5 m tall and 0.5 m wide as well as senescent plants, as identified by having over 50% of the stems dead or dying. This eliminated plants that might have too few growing shoots to be significantly galled by aphids, and those obviously departing from typical growth patterns in the population.

As a nitrogen source for enriching study plants, we applied granular urea fertilizer (46% nitrogen), widely used in agricultural research (Waring and Pitman, 1985; Thomas *et al.*, 1999). Urea is water-soluble and remains mobile in the soil until hydrolysed to ammonium. All treatments were applied on 14–15 May 2005, during wet weather and before the growing season, thus minimizing urea losses due to ammonia volatilization (Overdahl *et al.*, 1991). We applied 25 g urea dissolved in 2 L water to the soil for the 'low treatment' plants; 'high treatment' plants received 50 g urea in 2 L water; the control group received 2 L water only. In all instances, solutions were applied carefully, on the uphill side of each plant but within 0.5 m of its base, so as to maximize soil absorption while minimizing the potential for runoff. Because of the abundant ambient precipitation, we assumed the addition of 2 L water had only a negligible effect on growth of the host plant. Selected fertilizer concentrations were in accordance with standard protocols (*Forest Fertilization Guidebook*, 1995). Soil rather than foliar applications were chosen because *A. patula* absorbs nutrients quickly via its mycorrhizae (Largent *et al.*, 1980; G. Boivin, personal communication); the nitrogen content of soil is known to affect the nitrogen concentrations of foliar tissues (Dixon, 2005). Some study plants, either for lack of producing new growth or because of apparent resistance to herbivore

attack discovered after the application of fertilizer treatment, displayed no evidence of galls (galls otherwise persist 1–2 years). Consequently, we used only 12 of the 16 original control plants, 12 plants for the low treatments, and 11 plants for the high treatments.

As a means of evaluating host-plant quality, and to assess if plants were responding to nitrogen enrichments, foliar tissue samples were collected from all study plants. Because foliar nitrogen concentrations may vary seasonally (Karley *et al.*, 2002), we collected samples three times over the growing season: on 5 June 2005 we sampled leaf tissue produced the previous year, and on 19 August and 1 October 2005 we sampled new leaf tissue. As plants may respond to nitrogen treatments over multiple growing seasons (Waring and Pitman, 1985; Mopper and Whitham, 1992), we obtained a stratified sample of newly emerging leaf tissue from six plants on 3 August 2006 as well. (In *A. patula*, new growth is distinguishable from older foliage by the presence of glandular hairs.) Leaf tissue samples were stored at −80°C, then dried for 24 h at 60°C. Analysis for total percent nitrogen by weight was conducted by Olsen's Agricultural Laboratory (McCook, NE) using the Dumas Method (Moore *et al.*, 2010).

Plants with abundant inflorescence galls were sampled systematically from all sides of each bush; when scarce, all suitable galls were taken. We collected well-established but immature galls only, to avoid possible bias by failing to count aphids already exiting galls [immature galls are intact, lacking the exuvia otherwise present outside mature galls, from which adult aphids have emerged (Miller, 1998)]. We dissected as many galls as were available for each plant, up to a maximum of 14. We excluded galls lacking foundresses and those occupied by multiples of them.

We scored treatment sex ratios by dissecting galls and recording totals of males and sexual females in each brood. In the sexual generation of *T. coweni*, third- and fourth-instar males are distinguishable from females by their distinctly paler colour, smaller size, and genital armature (Miller and Avilés, 2000). These traits are less distinct in earlier developmental stages; hence, galls lacking third- or fourth-instar juveniles were excluded from the analysis. The proportions of first- and second-instar juveniles otherwise difficult to sex were assumed to follow the established sex ratio of the third- and fourth-instar juveniles within each brood. This assumption is likely valid because *T. coweni* does not temporally separate production of males and females within broods: emergence of adult males and females overlaps broadly and is usually completed within 6 days (Miller and Avilés, 2000). Estimates of relative brood size were obtained by counting only third- and fourth-instar aphids present at the time of dissection. Because the developmental period between larval birth and adult emergence is brief, this estimate serves as a good proxy to total production per brood. After assessing brood sex ratios, we stored the contents of each gall in 80% ethanol. By combining all broods from a given treatment into a single opaque vial, we simplified randomizing the subsequent selection of animals from across all plants and all broods for later analysis of mean dry weight and ova production.

To estimate potential changes in larval size in response to nitrogen enrichment treatments, we calculated mean dry larval mass. We attempted to reduce any confounding effect of body composition and mass ratio by defatting all animals in 80% ethanol, as female bias may otherwise be overestimated (Trivers and Hare, 1976; Boomsma, 1989). Thus the estimated mass of our samples reflects all remaining tissues such as protein, chitin, and other carbohydrates. Using an eye dropper, we selected 30 fourth-instar males and 30 fourth-instar females randomly from each treatment, then dried them for 24 h at 40°C in an incubator. We used a Cahn 29 automatic electrobalance (accuracy ± 0.001 mg) to weigh aphids. To compensate for possible loss of accuracy at the detection limits of the scale, we weighed the animals

in groups of five. Six such groups were weighed for a total of 30 individuals from each treatment and sex. Individual weights were then estimated from the group means. To verify the manufacturer's claimed precision of the electrobalance, all six groups from a given sex and treatment were weighed together. Differences in weight between the cumulative total of all six groups and the total weight of the 30 combined individuals on the scale were negligible (±0.003 mg per aphid).

To assess any differences between treatments in the number of ova produced by sexual females, we dissected and recorded the number of developing ova in a random sample of 30 fourth-instar females from each treatment. To reduce the potential of overlooking ova produced, only those females with mature ova (defined as those at least 0.5 mm long) were counted.

We used regression analysis to explore whether nitrogen enrichment treatments predicted the proportion of males in broods. Logistic regression, analysis of variance (ANOVA), and the *z*-test for differences among groups were employed for statistical analysis of sex ratio and aphid body mass: we evaluated variation at the levels of treatment, plant, and brood. We used the non-parametric Kruskal-Wallis test for comparing number of ova produced in the fourth-instar females and foliar nitrogen concentrations among treatments. We checked for homogeneity of variances in the data sets using residual plots; we transformed data as needed and examined them for normality. When ANOVA revealed significant differences among treatments, we applied the Tukey-Kramer test for multiple comparisons. We used the statistics package JMP 4.0 (SAS Institute, 2006) for logistic regression, ANOVA, and for multiple comparisons.

RESULTS

We examined 269 galls and 5800 aphids in total; among all treatments, broods averaged 38.62% male. Broods obtained from plants treated with nitrogen were significantly more female-biased than controls (two-tailed *z*-test: $z = 5.757$, $P < 0.005$).

Variation in sex ratio at the level of individual plants was consistent with treatment-wide results. The percentage of male aphids from plants receiving low or high nitrogen treatments was significantly lower than the controls (pooled data; one-way ANOVA: $F = 7.540$, $P = 0.0039$) (Fig. 1). We used arcsine transformation (in degrees) to render the data closer to normal distributions. Comparison of means showed differences were significant between the controls and both nitrogen treatments (Tukey-Kramer test: $P < 0.05$), but not between low and high treatments.

We applied logistic regression to develop a model predicting the proportion of male aphids in broods by treatment, and to determine the percent of variance explained by the treatments. As mean sex ratios per plant and per brood did not differ between the low and high treatments, these values were pooled. We compared the combined nitrogen treatment data ($n = 176$ broods) to the controls ($n = 93$) in this model. The proportion of male aphids per brood (arcsine transformed, in degrees) was plotted against treatment and weighted by brood size. Best fit of the model was determined with maximum likelihood using a modified Newton-Raphson iterative algorithm: weights were incorporated in calculation of the regression parameters. Differences between treatments in average estimated brood size were not significant (one-way ANOVA: $F = 1.853$, $P = 0.158$), although the controls yielded the smallest mean brood size (20.51 ± 1.25 (S.E.), $n = 93$ broods) with the low (22.25 ± 1.28, $n = 89$ broods) and high (23.97 ± 1.29, $n = 87$ broods) treatments progressively larger.

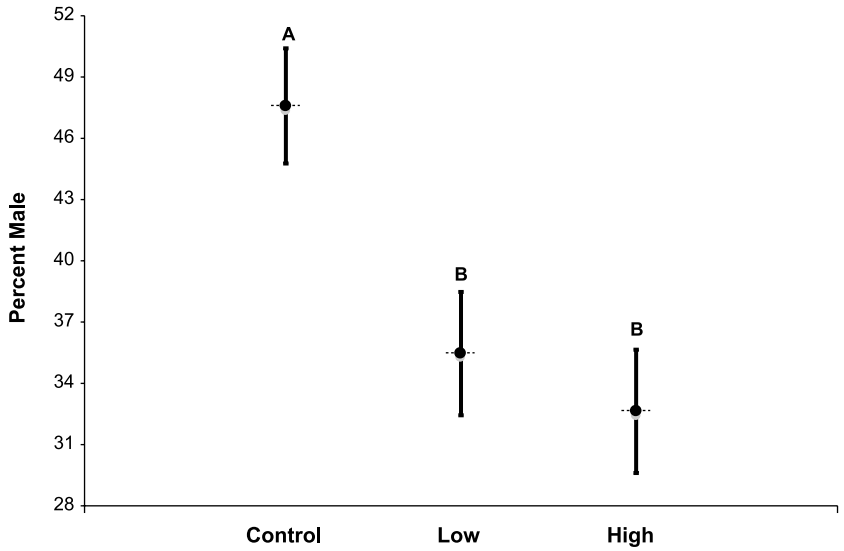

Fig. 1. Mean (±S.E.) percent male offspring on a per plant basis. Letters above dots indicate significant differences. High treatment plants ($n = 7$) received 50 g urea in 2 L water; low plants ($n = 7$), 25 g urea in 2 L water; controls ($n = 8$), 2 L water only.

Goodness-of-fit analysis of the logistic regression model showed it predicted the proportion of males in broods based on treatment, although it explained little of the variation in the data ($R^2 = 0.033$; $\chi^2 = 243.49$, $P < 0.001$). Because our model was unable to explain variance effectively, we tested the significance of the unexplained variance and the model's overall significance. A likelihood ratio test suggested our model fit the above results well (LR $\chi^2 = 243.49$, d.f. = 1, $P < 0.0001$). To evaluate the effect of combining our low and high treatments, we ran the model using just control and high treatment data, yielding a good fit, despite a low R^2 value ($R^2 = 0.048$; $\chi^2 = 264.27$, $P < 0.001$).

Pooled data on dried biomass of aphids showed no significant differences among treatments for either males (one-way ANOVA: $F = 1.78$, $P = 0.202$) or females (one-way ANOVA: $F = 0.25$, $P = 0.783$). Statistically significant differences were identified only between males and females (one-way ANOVA: $F = 67.07$, $P < 0.0001$) (Tukey-Kramer test, $P < 0.05$) (Fig. 2). Females averaged 2.0 times the size of males; since the pooled sex ratio was in excess of 61% females, the overall investment ratio exceeded 75% to females.

The number of presumptive ova carried by fourth-instar females revealed no significant differences in ova production among treatments (Kruskal-Wallis test: $\chi^2 = 4.636$, $P = 0.098$): control means were 7.70 ± 0.098 (S.E.) ($n = 30$ aphids); for the low treatment 7.90 ± 0.088 ($n = 30$ aphids); and for the high treatment 7.67 ± 0.1 ($n = 30$ aphids). The frequency distribution of these data was skewed to the left (Fig. 3). Analysis of quantile distribution in presumptive ova production confirms this trend.

Our study plants varied considerably in percent nitrogen by leaf dry weight within treatments, although assessments of foliar nitrogen concentrations did not reveal significant differences among treatments. Old-growth leaf tissues collected on 5 June 2005 contained, predictably, slightly less foliar nitrogen than new leaf tissues from all other sampling

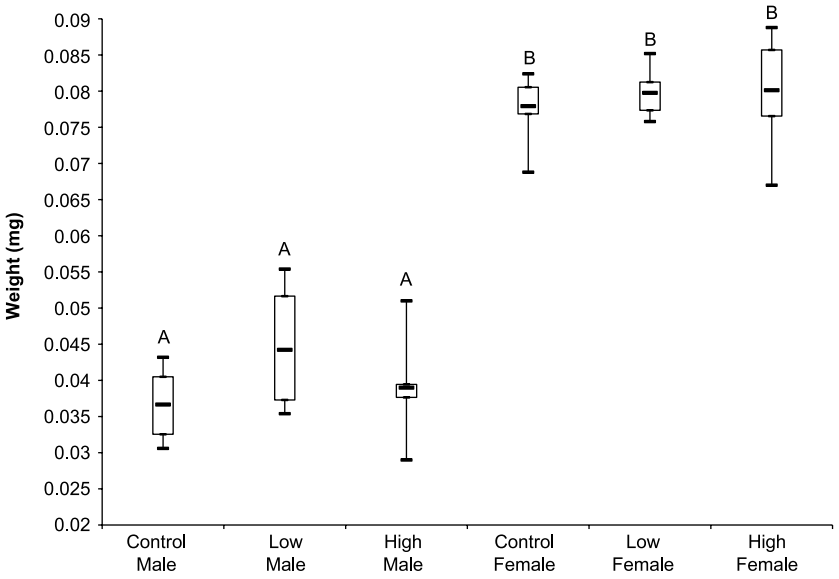

Fig. 2. Box plots of data for aphid dry weights by sex and treatment ($n = 30$ animals for each group). Letters indicate significant differences. Individual dry weight based on mean of five animals from six different groups.

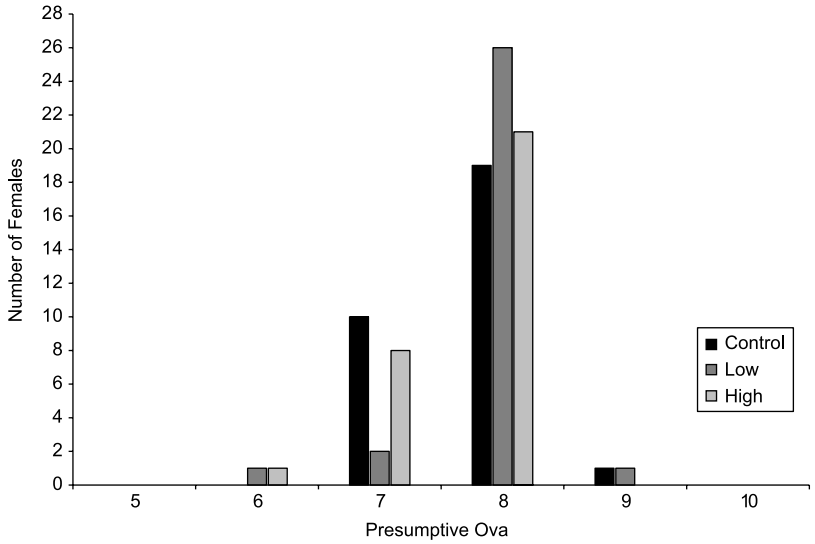

Fig. 3. Frequency distribution of presumptive ova in fourth-instar female larvae by treatment. Among all treatments mean number of ova present was 7.8.

periods, although we identified no significant differences among treatments (Kruskal-Wallis test: $\chi^2 = 1.300$, $P = 0.522$). Tissue samples from new leaves on all treatment plants collected on 19 August 2005 likewise suggested no significant differences (Kruskal-Wallis test: $\chi^2 = 0.243$, $P = 0.885$): control plants averaged $0.935 \pm 0.054\%$ (S.E.) ($n = 16$ plants) nitrogen

by weight; low treatment plants $0.921 \pm 0.073\%$ ($n = 16$) and high treatment plants $0.919 \pm 0.045\%$ ($n = 16$). The range of values within treatments was large for the control (0.65–1.41%), low (0.57–1.52%), and high (0.66–1.30%) treatments. These tissue samples were collected when gall formation on inflorescences had just commenced. Stratified samples across treatments of new leaf tissues collected on 1 October 2005 similarly revealed no statistical trends (Kruskal-Wallis test: $\chi^2 = 1.300$, $P = 0.522$; Kruskal-Wallis test: $\chi^2 = 1.559$, $P = 0.459$). The same held true for stratified samples of new leaf tissues collected on 3 August 2006 (Kruskal-Wallis test: $\chi^2 = 0.620$, $P = 0.733$): foliage from the control plants contained 0.913% nitrogen by dry weight ($n = 6$ plants); foliage from the low nitrogen plants had 0.978% ($n = 6$ plants), and foliage from the high treatment plants had 0.946% ($n = 5$ plants).

DISCUSSION

Our data indicate *Tamalia* aphid clones respond to a gradient of nitrogen enrichment of their host plants by skewing brood sex ratios increasingly towards female offspring. Treatment-wide results based on the total number of males and females show significant differences between the nitrogen enrichments and controls. Taken at the level of sex ratio on a per plant basis, our data reveal still more pronounced differences between treatments and controls. Hence our results solidly support the hypothesis that *Tamalia* aphids respond adaptively to enhanced resource availability. The control groups in our analyses show a slight but significant female bias, consistent not only with results from a prior study (Miller and Avilés, 2000), but with sex allocation patterns in other aphid species and a wealth of invertebrate systems (Dixon, 2005; West, 2009). Our logistic regression model further suggests *T. coweni* manipulates brood sex ratios according to resource quality, although it fell short of precisely explaining variance in allocation to male offspring within galls. This lack of precision is, however, consistent with the notoriously high levels of variability reported in a recent comprehensive review of sex allocation (West, 2009).

Perhaps surprisingly, *T. coweni* foundresses do not appear to respond to nitrogen enrichments by adjusting *per capita* body mass (Fig. 2): our data on dried aphid biomass revealed significant differences between the sexes, but not treatments. This stands in contrast with other published reports on aphid sex allocation, in which production of larger, more fecund individuals results from increased resource quality (Whitham, 1978; Dixon, 2005). Although the data presented here do not provide direct evidence that daughters accrue more fitness benefits from improved nutrition than do sons, prior empirical and theoretical work on aphid sex ratios, including that of *T. coweni*, supports this hypothesis (Gilbert, 1980; Moran, 1993; Miller and Avilés, 2000). In the present study, females were found to have significantly greater dry *per capita* mass than males, as expected. Because the animals were first defatted by storage in 80% ethanol, our calculated overall investment ratio, 75% to females, underestimates the true level of allocation to female offspring. As neither the mean number of presumptive ova *per capita* nor foundress mean mass varied by treatment, nitrogen enrichments appear to have no effect on the reproductive capacity of *T. coweni* foundresses. The observed female bias in broods consequently represents a significant preferential investment of resources by foundresses and may help to explain why these patterns are confined to manipulations of sex ratio rather than brood size, although the possibility remains that foundresses are ovule-limited. Again, it is important to bear in mind that any adaptive responses to enhanced environmental conditions represent investment decisions on a clonal,

not *per capita*, basis; hence the aphid genotype behaves as an 'evolutionary individual' (*sensu* Janzen, 1977).

Although our data suggest aphids indeed ingest supplemental nitrogen, the evidence is manifest more in the study animals than in the host plants. In a parallel, earlier experimental field study, foliar application of nutrients to leaves of the host plant *Populus fremontii* yielded significant increases in fecundity and body mass in the galling aphid *Pemphigus populivenae*, but not in leaf size of the host plant (Whitham, 1978). The distribution of *T. coweni* galls has been shown to be tightly correlated with the concentration of immature leaves on the host plant (Sholes and Beatty, 1987), underscoring a general pattern: the fastest-growing host-plant tissues predict the positioning of galls in a number of arthropod systems, including aphids (Dixon, 1998; Price, 2003). Because of their transient nature and great mobility, concentrations of nitrogenous compounds within plant tissues vary in both space and time (Saulnier and Reekie, 1995; Pugnaire, 2001; Karley *et al.*, 2002), potentially complicating their detection. Perhaps amino acid analysis, rather than total nitrogen content estimates of foliar tissue, may provide more biologically meaningful assays of nutritional content directly assimilated by *T. coweni*; however, such analysis poses significant technical challenges (Crawford *et al.*, 1995; Moore *et al.*, 2010). Because sex determination takes place in the embryonic stage of development, well before the offspring are born, the resulting lag may cause some species of aphid to respond to nutritional differences in host-plant quality more between, than within, generations (Ward and Wellings, 1994; Nevo and Coll, 2001). Finally, we cannot exclude the possibility that nitrogen enrichment resulted in an increase in total foliage production, rather than an increase in tissue nitrogen concentration, but we lack data on foliage production in the study plants. A detailed understanding of the growth patterns and factors affecting phytophagous insects such as *T. coweni* and its host plants will require continuous investigation over multiple seasons (Keeley and Keeley, 1977; Mopper and Whitham, 1992).

Extensions

Ecological data on invertebrates are inherently variable. As Whitham (1980) noted, theoretical models incorporating homogeneous habitats are not always directly comparable to empirical data from heterogeneous habitats. In a review of aphid sex ratios, Foster (2002) pointed out the potential utility of *T. coweni* as a system for investigating a mechanism for condition-dependent sex allocation. Here we have shown a naturally occurring population of the galling aphid *T. coweni* responds to nitrogen enrichment of the host plant through sex allocation in a manner predicted by theory. Few studies on sex allocation in wild populations have manipulated resource quality directly, as done here (Craig *et al.*, 1992; Komdeur and Pen, 2002). Still fewer such investigations of conditional sex allocation have involved diplo-diploid organisms, as compared to hymenopteran haplo-diploid systems (Clausen, 1939; Rosenheim *et al.*, 1996). Our findings, therefore, have significant implications for sex allocation theory within the context of optimizing investment decisions in clonal animals. Further trials spanning a wider range of nitrogen enrichment treatments may refine our understanding of the capacity of *T. coweni* for manipulating brood sex allocation. It would be useful to explore how the dynamics of resource availability across multiple parthenogenetic generations modulate investment patterns in the final, sexual generation. Because *T. coweni* foundresses frequently occupy galls communally (Miller, 1998), an additional dimension of sex allocation worthy of study will be to incorporate the consequences of social behaviours on sex allocation patterns in communal galls. These may include responses to increased

competition resulting from gall sharing, or conflict over sex allocation between unrelated foundresses (West, 2009).

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