

Sexually dimorphic body size and development time plasticity in *Aedes* mosquitoes (Diptera: Culicidae)

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ABSTRACT

Background: Sexual size dimorphism (SSD) in insects often accompanies a sexual difference in development time, sexual bimaturism (SBM).

Goal: To determine whether three *Aedes* mosquito species have similar plasticity in SSD, attain sexual dimorphism through similar strategies, and whether SSD and SBM are associated.

Organisms: *Aedes albopictus*, *Aedes aegypti*, and *Aedes triseriatus* (Diptera: Culicidae).

Methods: In four different food availability environments, we quantified plastic responses of relative growth rate (RGR), development time, and adult body size in individually reared males and females.

Results: Food availability affected RGR differently for the sexes for all three species. The RGR of males and females differed significantly in the 0.1 g/L food treatment. This difference did not account for observed SSD. Food levels over which the largest changes in RGR were observed differed among the species. Male and female adult mass and development time were jointly affected by food availability in a pattern that differed among the three species, so that degree of SSD and SBM changed differentially with food availability for all three species. Development time was generally less sexually dimorphic than mass, particularly in *A. albopictus*. At lower food levels, *A. aegypti* and *A. triseriatus* had accentuated dimorphism in development time. These results, combined with our knowledge of mosquito life history, suggest that a direct benefit of SBM is improbable for mosquitoes and that the observed intersexual differences in development time are more likely byproducts of selection for SSD.

Keywords: *Aedes aegypti*, *Aedes albopictus*, *Aedes triseriatus*, phenotypic plasticity, sexual bimaturism, sexual size dimorphism.

INTRODUCTION

Diverse taxa display sexual dimorphism, perhaps most commonly size dimorphism, wherein one sex is larger than the other. The ultimate cause of sexual size dimorphism (SSD) is divergent selection pressure on the sexes (Blanckenhorn, 2000; Stillwell *et al.*, 2010; Pyron *et al.*, 2013). Proximately, the sexes may attain different adult sizes by beginning life at different

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sizes, having different growth rates, developing for different lengths of time (Teder, 2014), or having different asymptotic sizes (Karkach, 2006) [i.e. the size at which an organism's growth stops or slows until trivial (Brown *et al.*, 1999)]. These different pathways to size dimorphism are not mutually exclusive and may act in concert to produce the observed sexual size dimorphism. Each strategy for growing to a greater size comes with costs, such as an increased risk of predation due to increased activity (e.g. foraging, to increase growth rate), or prolonged exposure to pre-reproductive mortality whatever the source, and it is likely that distinct intersexual differences in the balance of benefits and costs cause the variation in SSD seen across and within taxa (Stillwell *et al.*, 2010).

An important dissimilarity exists among ontogenies that achieve SSD via differences in initial mass, growth rate, development time, or asymptotic size. For the former two strategies, sexual size dimorphism will be produced even when the sexes reach maturity at the same time or age, resulting in SSD independent of sexual bimaturism (SBM), an intersexual difference in timing of maturity. In contrast, for the latter two, SBM is a necessary byproduct of SSD, a product of constraint on developmental mechanisms and selection for SSD. If initial size, growth rate, and asymptotic size are the same for males and females, SBM would result if males simply emerge at a lower proportion of the sexes' shared asymptotic size (Stamps and Krishnan, 1997). Alternatively, a sex difference in asymptotic size can produce sexual bimaturism when the sexes mature at the same proportion of asymptotic size, with the larger sex necessarily reaching its final size later (Stamps and Krishnan, 1997). It is also possible that SBM itself could be directly selected (Nylin *et al.*, 1993; Morbey and Ydenberg, 2001; Teder, 2014). For example, if the first females to mature are more fecund, if there is first-male sperm precedence, or if females mate only once, the early maturation of a male (protandry) would be highly advantageous. Plasticity in SSD or SBM could be the result of differential plasticity in body size or development time for males and females, and has the potential to be adaptive if the optimal phenotypic difference between males and females is condition dependent.

Alternative patterns of SSD among insects are often ascribed to some form of sexual selection such as territoriality [Odonates (Serrano-Meneses *et al.*, 2008)] or female reluctance [Ichneumonine wasps (Teder, 2005)], but can arise when differential effects of body size on reproductive fitness result in differential natural selection for size between males and females (Blanckenhorn, 2000). Fecundity selection on females probably accounts for female-biased SSD in most invertebrates, as fecundity increases strongly with adult weight (Honěk, 1993; reviewed by Blanckenhorn, 2000). Larger males may transfer more sperm than smaller males (e.g. Ponlawat and Harrington, 2007, 2009; Helinski and Harrington, 2011), which may account for some cases of male-biased SSD. Blanckenhorn (2000) noted that among the costs of attaining large size, delayed maturation, with greater exposure to pre-reproductive mortality and delayed reproduction, may be the most general and important. This observation suggests that for many organisms, plasticity in adult size and development time, and therefore potentially in SSD and SBM, is likely an adaptive response to optimize the costs and benefits of adult size (Nylin and Gothard, 1998; Uhl *et al.*, 2004; Rennie *et al.*, 2008). Like most insects (Stillwell *et al.*, 2010), *Aedes aegypti*, *A. albopictus*, and *A. triseriatus* exhibit female-biased SSD and protandrous SBM. *Aedes* also show considerable plasticity in growth rates, sizes, and development times (e.g. Costanzo *et al.*, 2011; Yee *et al.*, 2012), making them an excellent study system for exploring plasticity of growth in response to environmental differences. The goal of this study was to use experimental manipulations of food availability to quantify plastic responses of adult mass, development time, and growth rate, and to determine for these three species of *Aedes*

whether: (1) they attain SSD by the same mechanism; (2) they have similar plasticity in SSD; and (3) SSD and SBM are associated.

METHODS AND MATERIALS

Freely mating *Aedes albopictus*, *A. triseriatus*, and *A. aegypti* laboratory colonies originating in Florida, USA deposited eggs on seed germination paper. Egg papers were held in a humid environment for approximately one week before being dried and stored prior to hatching. To hatch eggs, we placed small strips of egg paper in vials with a suspension of Difco™ Nutrient Broth and water (0.4 g/L) for approximately one day. First instar larvae were then rinsed and placed in treatments.

Test larvae were reared individually in water-filled glass vials (20 mL) at 26°C and a 14:10 light/dark photoperiod, and fed on an aqueous suspension of yeast-lactalbumin (1:1) at four different concentrations: 0.05 g/L (Low), 0.1 g/L (Med–), 0.15 g/L (Med+), and 0.2 g/L (High). Every second day, we placed larvae in fresh food suspension of the same concentration to hold food level reasonably constant. We checked individuals daily for maturation, and recorded development time (days to adulthood) and determined sex. Adult mosquitoes were then dried at 50°C for approximately one week and adult dry mass determined to the nearest 0.1 µg. Mass was log transformed for analysis to meet the assumption of normality.

In determining growth rates, we assumed that male and female first instars hatch at the same mass, which is nearly universal in insects (Tammaru *et al.*, 2010). An exception is a vespid wasp, in which females determine the sex of offspring (Budriené *et al.*, 2013). In mosquitoes, mass is allocated to eggs prior to fertilization (Roth and Porter, 1964) and the male gamete determines offspring sex (Hickey and Craig, 1966). Thus, for *Aedes*, no obvious mechanism exists by which a female could produce size-dimorphic fertilized eggs. To determine first instar mass, we dried three replicate batches of ten newly hatched larvae each of *A. albopictus*, *A. triseriatus*, and *A. aegypti* at 50°C and weighed replicates on a Cahn C31 microbalance, to the nearest 0.1 µg, and calculated mean mass per larva. Growth rates were estimated for each individual as the instantaneous relative growth rate [RGR (Tammaru and Esperk, 2007)], which models growth as though larvae grow at a constant exponential rate across the entire growth period:

$$\text{RGR} = (\log(\text{adult dry mass}) - \log(\text{mean first instar dry mass})) / (\text{development time}).$$

Relative growth rate calculated in this way, over the entire developmental period, represents an average growth rate over development, because most insects have different growth rates for different instars, with lower RGRs in the ultimate larval instar and, more generally, growth rates that decrease with mass (Tammaru and Esperk, 2007). We use RGR here simply as a means of comparing the overall trajectories of growth for different species, sexes, and food levels. Differences in RGR among food levels, species, and sexes were examined using analysis of variance (ANOVA). Significant differences in growth rates were further resolved using Tukey's test and *post-hoc* contrasts. Growth rate was also estimated as the difference between log-transformed cube roots of adult and initial mass divided by development time, assuming a linear growth rate in length proportional to cube root of mass (as described by Tammaru and Esperk, 2007). ANOVA on this estimate of growth rate yielded the same statistical conclusions about significant effects of food, species, and sex, and is therefore not reported here.

Differences among combinations of sex, food level, and species were examined using multivariate analysis of variance (MANOVA) on adult dry mass and days to adulthood. *Post-hoc* multivariate contrasts were used to evaluate significant effects; standardized canonical coefficients [SCCs (Scheiner, 2001)] were used to quantify the contributions of dependent variables to significant differences among sexes, food levels, and species. Percent sexual size dimorphism and sexual bimaturism were calculated as (Blanckenhorn *et al.*, 2007):

$$100 \times \left[\frac{(\text{Male} - \text{Female})}{(\text{Male} + \text{Female})/2} \right]$$

RESULTS

Relative growth rate was significantly affected by species, food, and the interactions of sex $\times$ food and species $\times$ food (Table 1). The absence of a three-way interaction among sex, food, and species indicates that the effects of food on RGR of the sexes are statistically indistinguishable for all three species. Thus, the only significant effect involving sex indicates that the effect of food on RGR depended on sex (Fig. 1a). The RGR for females differed significantly between the Med+ and Med– food levels, but RGR for males differed significantly between the Med– and Low and between the Med+ and High food levels (Fig. 1a). This intersexual difference in responses to treatments was primarily driven by a significant difference, averaged across species, in RGR for males and females fed the Med– food treatment (Fig. 1a), although *post-hoc* contrasts within the species indicated that only in *A. aegypti* fed the Med– treatment did the different RGRs of the sexes attain statistical significance ($F_{1,361} = 16.88$, $P < 0.0001$), with males having a higher RGR than females at this food level (Fig. 2b). The lack of a three-way interaction likely arises because this same trend for intersexual difference in RGR in the Med– food treatment is present (if not individually significant) in all three species (Fig. 2b). *Post-hoc* power analysis for RGR data indicated that this test for a three-way interaction had an observed power of 0.437, and that to attain a power of 0.8 with the observed differences among means, we would have needed a total N of 735 individuals, almost twice the size of our sample. The differences

Table 1. Results of MANOVA testing effects of species, food, and sex on adult dry mass and development time (left), and ANOVA testing effects of the same factors on RGR (right)

Factor	MANOVA on dry mass and development time					ANOVA on RGR		
	d.f.	Pillai's trace	Pr > F	SCC mass	SCC days	d.f.	F-value	Pr > F
Species	4, 722	0.63707	<0.0001	1.236	1.365	2	297.55	<0.0001
Food	6, 722	0.62256	<0.0001	2.072	–0.276	3	73.44	<0.0001
Sex	2, 360	0.59215	<0.0001	2.072	0.590	1	1.76	0.185
Species $\times$ Food	12, 722	0.08988	0.0008	1.848	–0.661	6	2.58	0.0186
Species $\times$ Sex	4, 722	0.02445	0.0638	2.073	–0.272	2	1.35	0.26
Food $\times$ Sex	6, 722	0.03907	0.0266	–0.688	1.418	3	4.54	0.0039
Species $\times$ Food $\times$ Sex	12, 722	0.06078	0.0329	1.889	0.893	6	1.39	0.2172
Error						361		

Note: Significant effects are shown in **bold** print. SCC = standardized canonical coefficient.

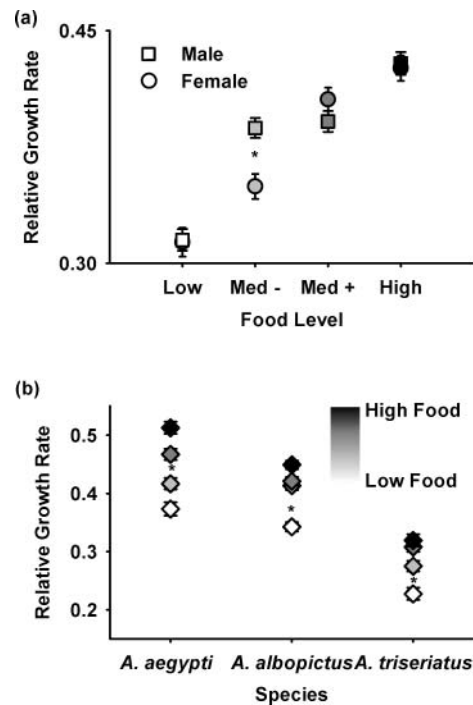


Fig. 1. Least squares means $\pm$ standard error for sex $\times$ food (a) and species $\times$ food (b) effects on relative growth rate. (a) Males grow faster than females with low-to-moderate food availability. (b) *Aedes aegypti* showed a greater RGR difference between the two moderate food treatments, whereas RGR for *A. albopictus* and *A. triseriatus* differed most between the two least food treatments. Asterisks indicate significant differences of adjacent least squares means.

among sex/food level/species means would have had to be 40% greater, with no change in variation, to have attained a power of 0.8.

Overall, *A. aegypti* had the highest RGR, *A. albopictus* one slightly lower, and *A. triseriatus* the lowest (Fig. 1b). The species $\times$ food interaction resulted from different patterns of increased RGR with increased food for the three species (Fig. 1b). *Aedes aegypti* had the greatest increase in RGR between Med+ and Med- food levels, whereas *A. albopictus* and *A. triseriatus* RGR increased most between the Low and Med- food levels (Fig. 1b).

MANOVA on effects of food level, species, and sex on adult mass and development time yielded significant effects of all factors and interactions except the species $\times$ sex interaction (Table 1). The standardized canonical coefficients for the three-way interaction (Table 1) indicated that significant differences among species/sex/food combinations were primarily driven by differences in mass, although development time contributed non-trivially to this significant effect. Contrasts testing intersexual differences in all possible species/food level combinations were significant for all species at all food levels, demonstrating that the sexes did not converge in mass or development time at any food level tested. In all groups, standardized canonical coefficients indicated that mass was the primary dependent variable contributing to significant differences between the sexes (Table 2). For only two groups was the contribution of development time to intersexual differences comparable in magnitude to

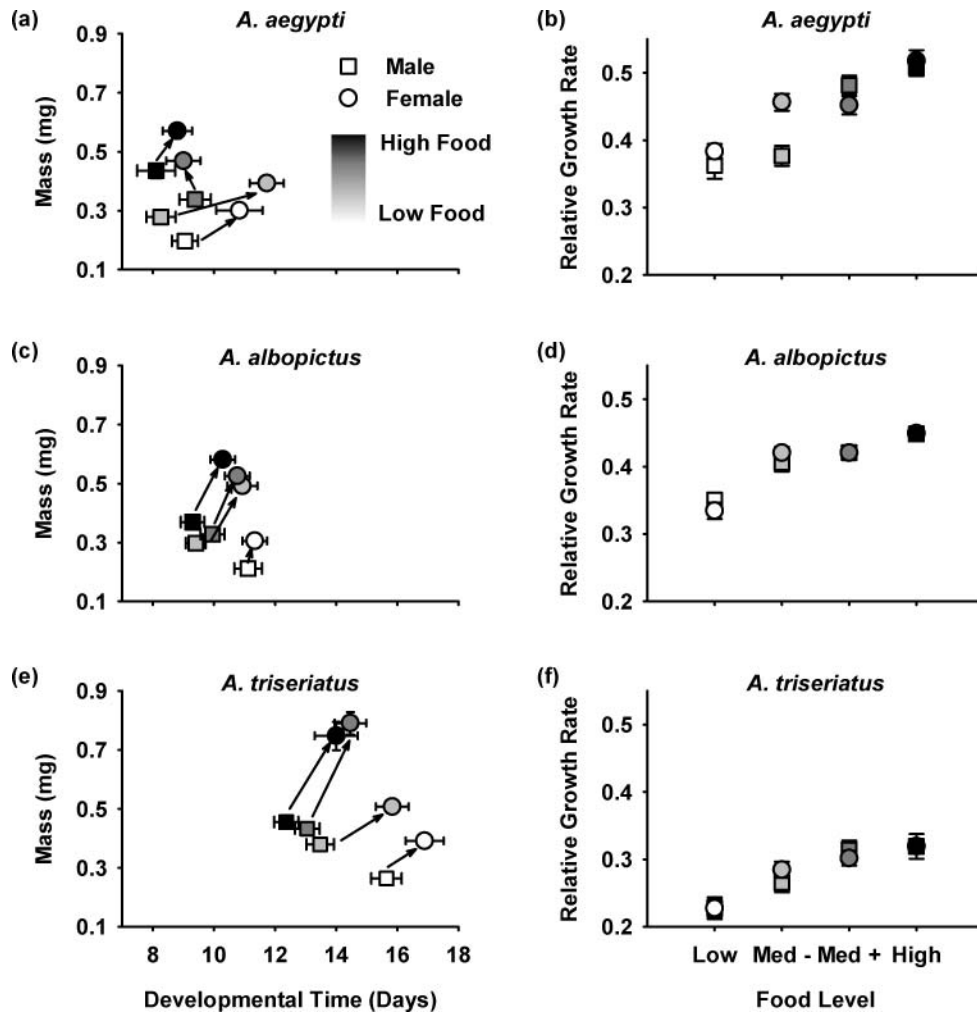


Fig. 2. Least squares means $\pm$ standard error for RGR (right), and for adult dry mass and development time (left) for species $\times$ sex $\times$ food combinations. For RGR, in food treatments resulting in differences between the sexes, whether significant or not, the difference is in the same direction (female greater or male greater) but the magnitudes of the differences vary. For the multivariate differences in size and development time, species achieved those differences in dissimilar ways. Arrows point from male to female within food treatments to enhance interpretability.

that of mass: Med– for *A. triseriatus* and *A. aegypti* (Fig. 2a,e). Among species, sexual dimorphism in mass and development time was significantly affected by food level for *A. aegypti*, but not for *A. albopictus* or *A. triseriatus* (Table 3). For *A. aegypti*, the effect resulted primarily from differences in development time (Table 3, SCCs; Fig. 2a). For *A. aegypti*, but not for the other two species, food levels produced a much higher percent SBM than percent SSD (Fig. 3), but that change in SBM did not trend monotonically with food. Rather, intermediate food levels (Med+, Med–) produced the most extreme SBM (Fig. 3).

Table 2. Multivariate contrast results for comparing the sexes within species–food combinations for mass at and days to adulthood

Group	$N_{\text{♀}}, N_{\text{♂}}$	d.f.	Pillai's trace	Pr > F	SCC mass	SCC days
<i>A. aegypti</i> Low	6, 19	2, 360	0.0831	<0.0001	2.0145	0.7079
<i>A. aegypti</i> Med–	11, 15	2, 360	0.1254	<0.0001	1.6343	1.1363
<i>A. aegypti</i> Med+	11, 13	2, 360	0.0537	<0.0001	2.1442	–0.0112
<i>A. aegypti</i> High	15, 9	2, 360	0.0397	<0.0001	2.1009	0.5124
<i>A. albopictus</i> Low	21, 17	2, 360	0.1043	<0.0001	2.1540	0.2481
<i>A. albopictus</i> Med–	14, 32	2, 360	0.2021	<0.0001	2.0790	0.5724
<i>A. albopictus</i> Med+	21, 22	2, 360	0.1868	<0.0001	2.1311	0.4040
<i>A. albopictus</i> High	21, 23	2, 360	0.1816	<0.0001	2.1160	0.4636
<i>A. triseriatus</i> Low	9, 14	2, 360	0.0804	<0.0001	2.0720	0.5904
<i>A. triseriatus</i> Med–	12, 17	2, 360	0.0864	<0.0001	1.7609	1.0295
<i>A. triseriatus</i> Med+	13, 21	2, 360	0.2235	<0.0001	2.1088	0.4880
<i>A. triseriatus</i> High	22, 7	2, 360	0.1215	<0.0001	2.0666	0.6023

Note: Bonferroni corrected critical $\alpha_c = 0.00417$. Standardized canonical coefficients (SCC) show the relative importance of mass and days in contributing to sexual dimorphism. Higher SCC values indicate a greater contribution to the observed dimorphism in species–food combinations.

Table 3. Multivariate contrasts testing the effect of food availability on sexual dimorphism within each species

Group	d.f.	Pillai's trace	Pr > F	SCC mass	SCC days
<i>A. albopictus</i>	6, 722	0.0166	0.4194	1.6843	1.0968
<i>A. aegypti</i>	6, 722	0.0447	0.0119	0.5542	1.5409
<i>A. triseriatus</i>	6, 722	0.0345	0.0381	2.0962	–0.2091

Note: Bonferroni corrected $\alpha_c = 0.01667$. Standardized canonical coefficients (SCC) show the relative contribution of mass and days in significant effects. Higher values indicate a greater contribution to changes in dimorphism across food treatments.

DISCUSSION

Patterns of growth can vary widely both across and within species even when environments are similar (Fairbairn *et al.*, 2007). Because body size is an important determinant of reproductive success in many systems, it becomes important to understand the sources of size variation and their consequences. Mosquitoes, like other insects, are most likely sexually monomorphic in size as propagules (see Tammaru *et al.*, 2010, and citations therein), and our data indicate that RGRs for the sexes are either statistically indistinguishable, or, if significantly different (Med– food level, Fig. 1a), inadequate to account fully for the observed SSD, because the smaller males had significantly greater growth rate. This finding suggests that a sexual difference in development time or asymptotic size contributes to SSD in these species. Indeed, when both growth rates and asymptotic sizes differ between the sexes, the smaller sex typically has the higher growth rate but lower asymptotic size (Stamps, 1993), and a similar pattern may be seen when both development times and growth rates differ. Still, differential plasticity in RGR in response to food availability may contribute to the observed change in

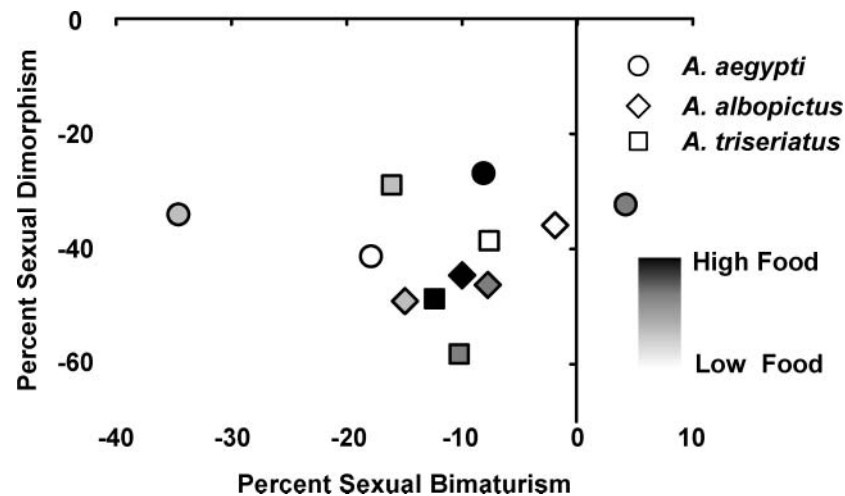


Fig. 3. Relative SSD and SBM expressed as percent sexual size dimorphism and percent sexual bimaturism for different food level treatments and species. SSD and SBM are highly plastic for all species, although *A. aegypti* varies more than the other two species (see also Table 3).

degree of SSD and SBM across food treatments. In the Med– food treatment, growth rates of males were significantly higher than those of females (Fig. 1a), especially for *A. aegypti* (Fig. 2b), a difference that appears to be mediated more by changes in development time than adult mass (Table 1). Although temperature is an important determinant of developmental rate variability in *A. aegypti* (Couret and Benedict, 2014), resource availability appears to play a role as well, and resources affected the sexes differently under some conditions. The differential growth rate plasticity of male and female *A. aegypti* may contribute to their more variable changes in sexual dimorphism and sexual bimaturism across food environments compared with the other two species of *Aedes* (Table 1, Figs. 2, 3).

The differential response to food availability between the sexes suggests intersexual differences in metabolism or foraging behaviour, with males in the Med– food treatment appearing more efficient at converting food in the environment into body mass, speeding their development. Intersexual differences in foraging strategy have been investigated in many taxa, often with a specific focus on sexual size dimorphism (e.g. Shine, 1991; Main *et al.*, 1996; González-Solis *et al.*, 2000; Blanckenhorn, 2005; Page *et al.*, 2005). For mosquitoes, intersexual differences in behavioural response to predator cues have been demonstrated in *Aedes triseriatus*: in the presence of cues to predation, males – the smaller sex – reduce the proportion of time spent in risky behaviours, including foraging, to a greater degree than do females, especially when hungry (Wormington and Juliano, 2014). Whether this behavioural difference leads to or is a result of sexual size dimorphism or sexual bimaturism remains unresolved. However, it is likely that nutritional effects on physiological correlates of sexual dimorphism (e.g. a difference in needs for growth and reproduction) affect the sexes differently, leading to differential plasticity in growth rates and, therefore, degree of SSD or SBM (Uhl *et al.*, 2004; Rennie *et al.*, 2008). The three species of mosquito in this study show different patterns of plasticity of SSD and SBM in response to different feeding environments. *Aedes aegypti* shows the greatest plasticity of both absolute (Fig. 2a) and relative (Fig. 3) SBM, with maximum protandry at low food levels (Figs. 2a, 3). In contrast, *A. albopictus* shows

very consistent SSD and SBM, except at the lowest food level where SSD and SBM are minimal (Figs. 2c, 3). Sexual size dimorphism is minimal for *A. triseriatus* at lower food levels (Fig. 2e) and protandry is least at the lowest food level (Figs. 2e, 3). The complexity of the plastic responses of these three ecologically similar species suggests complex interspecific differences in physiology and life histories.

Sexual dimorphism can provide clues to the mating ecology of an organism, and vice versa, through our knowledge of macroevolutionary patterns of SSD (Kraushaar and Blanckenhorn, 2001; Ding and Blanckenhorn, 2002; Fairbairn *et al.*, 2007; Allen *et al.*, 2014). Modest differences in mating system, such as strength of sexual selection, can result in measurable differences between species in sexual size dimorphism (Fairbairn *et al.*, 2007). Among or within species, SSD is produced through genetic, developmental, and physiological processes interacting with environmental factors such as resource availability, temperature, or mortality risk to yield unique growth trajectories (Fairbairn *et al.*, 2007). Body size at – in addition to the timing of – life-history events such as adulthood can be under selection, and the optimal phenotype, or, in the case of SSD and SBM, phenotypic difference, depends in large part on the competitive environment faced by the adult. Some aspects of the growth environment will be correlated with ecological factors influencing an adult's fitness, whereas others will not. In situations where fitness in the adult environment is closely predicted by conditions in the growth environment, we might expect to see a more consistent, canalized response of adult phenotype to environmental differences during development.

Because generations of wild mosquitoes overlap and female mosquitoes may mate multiple times (Boyer *et al.*, 2012; Helinski *et al.*, 2012), a shorter development time for males is unlikely to provide a strong mating advantage except at the beginning of the breeding season when sexually mature mates are rare (Singer, 1982). Instead, a correlate of development time, such as adult body size, may provide a selective advantage leading to female-biased SSD (Teder, 2014). Aerial courtship or coercion requires agility, a quality complexly related to body mass (Dudley, 2002). Males of intermediate body size and energy reserves were found to be more likely to obtain mates in the swarming *Anopheles gambiae* compared with both larger and smaller males (Ng'habi *et al.*, 2008), suggesting stabilizing selection at work on male body size in some mosquitoes. Although not on the same scale as *Anopheles* and not strictly required for reproduction (Oliva *et al.*, 2014), on-the-wing mating aggregations of *A. aegypti* (Cator and Harrington, 2011) and *A. albopictus* (Bellini *et al.*, 2010) have been observed in nature; similar aggregations have been observed in the laboratory for *A. triseriatus* (Foster and Lea, 1975). The relative reproductive success of large and small males of these *Aedes* species has not been explored, although there is mounting evidence of female mate choice based on male wing beat frequency in *A. aegypti* (Cator and Harrington, 2011). A better grasp of the relationships of body size to this and other factors influencing mate choice, mating frequency, and reproductive success could enhance our understanding of how ecological factors impact evolution of SSD and SBM in these and other species. Proximately, if a difference in development time for the sexes is indeed a byproduct of selection for sexual size dimorphism in insects (Teder, 2014) and other taxa, future studies on the proximate causation of SSD may benefit from the consideration of a difference in asymptotic size, a component of growth curves largely ignored by SSD studies, in addition to a simple difference in development time.

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