

Sex ratio bias in the dung beetle *Onthophagus taurus*: adaptive allocation or sex-specific offspring mortality?

Clarissa M. House · Leigh W. Simmons · Janne S. Kotiaho ·
Joseph L. Tomkins · John Hunt

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Abstract Sex allocation theory predicts that females should adjust the sex of their offspring when the fitness returns of one sex are higher than the other. However, biased sex ratios may also arise if mortality differs between the sexes. Here, we examine whether offspring sex ratio bias in the dung beetle, *Onthophagus taurus*, represents adaptive sex allocation by females or is due to sex-specific mortality. First, we re-analyze an existing data set to show that females produce an excess of daughters when mating to smaller, less attractive males and near equal sex ratio with large, more attractive males. We show, that this results from females adjusting larval provisions after mating to males of variable attractiveness which in turn influences the likelihood that sons die during development. Second, we conduct a manipulative experiment varying the quantity and quality of larval provisions and show that the mortality of sons increased when larval provisions were reduced. Collectively, our work demonstrates that offspring mortality is contingent on the amount of resources provisioned by females and that sons have greater nutritional demands than daughters during development, leading to higher mortality. Our results therefore demonstrate the importance of considering sex-specific offspring mortality in studies of sex ratio evolution.

Keywords Sex ratio · Sex-specific mortality · Nutritional resources · *Onthophagus taurus*

C. M. House · J. Hunt (✉)
School of Biosciences, Centre for Ecology and Conservation,
The University of Exeter in Cornwall, Penryn TR10 9EZ, UK
e-mail: J.Hunt@exeter.ac.uk

C. M. House
e-mail: J.Hunt@exeter.ac.uk

L. W. Simmons · J. L. Tomkins
School of Animal Biology (M092), Centre for Evolutionary Biology,
The University of Western Australia, Nedlands, WA 6009, Australia

J. S. Kotiaho
Department of Biological and Environmental Sciences, Centre of Excellence in Evolutionary Ecology,
University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

Introduction

Fisher (1930) showed that when parental expenditure in male and female offspring is the same, an equal sex ratio should evolve through natural selection. However, many species of insects (Charnov et al. 1981) and some vertebrates (Komdeur and Pen 2002) exhibit skewed offspring sex ratios. Sex allocation theory predicts that females should adjust the sex of their offspring when the fitness returns from one sex are higher than those from the other (Trivers and Willard 1973). In particular, factors such as local mate competition (Hamilton 1967), resource competition (Clark 1978), mate attractiveness (Burley 1981, 1986), maternal body condition (Trivers and Willard 1973), and variable environments (Charnov et al. 1981), have been shown, in theory, to influence how a mother should adjust the sex ratio of her offspring. While various empirical studies in insects (West and Sheldon 2002), birds (Komdeur and Pen 2002; West and Sheldon 2002), and ungulates (Hewison and Gaillard 1999) provide clear support for sex allocation theory, numerous inconsistencies still remain (e.g. Cameron 2004; Ewen et al. 2004; Fawcett et al. 2007).

Biased offspring sex ratios may also arise in the absence of adaptive sex allocation if one sex is more susceptible to mortality than the other. This may occur if one sex has higher growth rates and/or longer development times, making the sex more vulnerable to nutritional stress or disease (Clutton-Brock and Iason 1986). If differential mortality of the sexes occurs under some environmental or social conditions, but not others, this may partially explain the inconsistent support for theories of adaptive sex allocation. This is particularly likely to be a problem in empirical studies that examine sex ratio bias in offspring after substantial mortality has already occurred (Cockburn et al. 2002). Therefore, before any bias in offspring sex ratio can be considered adaptive, empirical studies must account for any possible sex differences in the mortality of offspring (Cockburn et al. 2002). This can be achieved either by determining the sex of offspring prior to major mortality events using molecular techniques, or by directly quantifying sex-specific mortality through experimental manipulation (Cockburn et al. 2002).

Dung beetle life-history

Male dung beetles (*Onthophagus taurus*) are dimorphic in their body plan, with large “major” males developing elongated head horns and small “minor” males remaining essentially hornless (Hunt and Simmons 1997; Tomkins et al. 2005). This male dimorphism is associated with alternative mating tactics, with major males fighting for access to females and minor males sneaking copulations (Moczek and Emlen 2000). In natural populations, beetles occur at high density (3–5,000 beetles in a 3L dung pad) with an equal sex ratio but minor males typically exceed major males by a ratio of 4 to 1 (Hunt et al. 1999). Controlled experiments in laboratory populations with the above composition have shown that there is a nonlinear increase in fitness with body size across the male morphs (Hunt and Simmons 2001). The reproductive success of major males is, on average, 4.5 times higher than that for minor males and increases linearly with body size. The reproductive success of minor males is unrelated to their body size (Hunt and Simmons 2001). In contrast to males, female fitness components (i.e. fecundity and survival) increase linearly across the natural range of female body sizes when females provision in isolation (Hunt and Simmons 2002). We do not currently know, however, whether the increase in fitness with size is greater in males or in females when measured under the same conditions, but we suspect the latter is more likely due to intense sperm competition in this species (Simmons et al. 1999).

During reproduction, females excavate dung from the dung pad, construct brood masses, and lay a single egg into each brood mass. One brood mass thus represents the entire food source for the development to adulthood of a single larva (Halffner and Edmonds 1982). Consequently, brood mass weight is a major determinant of offspring size, and subsequent fitness, in this species (Hunt and Simmons 1997, 2000, 2004). Indeed, physiological experiments have shown that horn development in *O. taurus* is regulated by juvenile hormone (JH), the release of which is triggered by the amount of dung consumed by developing larvae (Emlen and Nijhout 1999). Minor males consume less dung during development and receive a pulse of JH that inhibits horn development, whereas major males consume more dung and do not receive this pulse of JH permitting the development of elongated horns (Emlen and Nijhout 1999). The amount of dung that a female provisions in a brood mass is known to exhibit a high degree of plasticity in *O. taurus* (Hunt and Simmons 2000, 2004; Kotiaho et al. 2003). In particular, females produce lighter brood masses when provisioning on higher quality horse dung compared to low quality cow dung (Hunt and Simmons 2004), and produce more, heavier brood masses when mated to larger males (Kotiaho et al. 2003). Offspring sex ratios have been observed to covary with plasticity in brood mass weight, raising the possibility that females may adjust the sex ratio of their offspring (Kotiaho et al. 2003; Kotiaho et al. unpublished). However, offspring mortality is also dependent on the weight of the brood mass (Hunt and Simmons 1997, 2004), and if mortality is sex-specific in *O. taurus*, this may also explain any bias in offspring sex ratios.

Here we examine whether female *O. taurus* adjust the sex ratio of their offspring depending on the attractiveness of their mate and/or the quality of the resources used to provision offspring. We start by re-analyzing the data from Kotiaho et al. (2003) to determine if the increase in brood mass weight after mating with a large male is associated with a bias in the sex ratio of offspring. We then conduct an experiment where we manipulate the weight and nutritional quality of brood masses at random with respect to the sex of the larvae developing within brood masses, and examine the sex ratio of adult offspring that emerge. Although the fact that sex ratio variation in emerging offspring exists in *O. taurus* raises the possibility that parents are able to manipulate the sex ratio of offspring, the complexity of this system makes it difficult to make clear predictions about whether it is adaptive for mothers to produce an excess of male or female offspring when partner attractiveness and resource quality varies. For example, depending on exactly how the rate at which fitness increases with size in the sexes, it is likely that an adaptive explanation could be provided to support any possible outcome in offspring sex ratio. We can, however, make a clear prediction about the effect that differential mortality should have on offspring sex ratio bias in *O. taurus*. If offspring mortality is sex-specific, we predict a sex ratio bias in favour of the sex with the lowest mortality and that this bias will be associated with differences in the weight and quality of brood masses provisioned by the female.

Materials and methods

Collection of beetles

Several thousand *Onthophagus taurus* were collected from dung pads in Margaret River in the southwest of Western Australia. Beetles were maintained in the laboratory at 25°C in mixed sex cultures for 2 weeks with constant access to fresh cow dung to ensure that all beetles were reproductively mature and mated.

Offspring sex ratio and mate quality

To examine the relationship between offspring sex ratio and mate attractiveness, we re-analyzed the genetic data set of Kotiaho et al. (2003). In brief, 50 field-collected sires were taken at random from the culture, and each was housed with three F_1 laboratory-reared virgin dams for 3 days to mate. Dams were randomly allocated to sires and there was no difference in the pronotum width of dams across sires ($F_{49,150} = 0.41$, $P = 1.000$). Mated dams were established in individual breeding chambers (PVC piping 30 cm in length and 9 cm in diameter) three-quarters filled with moist sand and provided with 250 ml of fresh cow dung. After 7 days, all brood masses were collected (19.36 ± 0.21 per dam; total $n = 2,904$ brood masses), excess sand removed, and weighed individually to the nearest 0.01 g with an electronic balance. Brood masses were then placed in individual sand-filled chambers. At emergence, we determined the sex of each offspring. One week after the last beetle emerged, we recorded those brood masses that did not produce surviving offspring. This was further verified by individually opening each brood mass and inspecting the brood chamber.

Offspring sex ratio and resource quality

To examine the relationship between offspring sex ratio at emergence and the environment experienced by larvae during development, we experimentally manipulated brood mass weight and the quality of dung contained in the brood mass in a two-way factorial design. We randomly selected 400 females from the mixed sex culture and established them in individual breeding chambers, half being provided with 250 ml of horse dung (high dung quality) and half with 250 ml of cow dung (low dung quality). Chambers were maintained for 7 days and all brood masses produced on each dung type were collected and placed in a large individual container. A total of 800 brood masses were then drawn at random from each of these two containers and half of the brood masses provisioned on each dung type were randomly assigned to either the brood mass reduction treatment or the control treatment. In each of our four treatment combinations we therefore had 400 brood masses (total $n = 1,600$ brood masses). Excess sand was removed from each brood mass using a dissecting probe. Brood masses in the control treatment remained at their original weight. Brood masses in the manipulated treatment had their weight experimentally reduced, by a minimum of 25%, by removing a portion of the brood mass distal to the brood mass chamber (containing the developing larvae) using a scalpel blade (Hunt and Simmons 1997). Each brood mass was then individually weighed to the nearest 0.01 g and buried in moist sand in an individual container ($5 \times 5 \times 5$ cm) for larvae to develop. After emergence, we recorded the sex of the offspring produced and we recorded which brood masses did not produce viable offspring.

Statistical analysis

To determine if females adjust the sex ratio of their offspring with the attractiveness of their partner, we compressed the data set of Kotiaho et al. (2003) to sire means. The average number of brood masses produced by the 3 dams mated to each sire was 58.08 ± 0.79 . We calculated the sex ratio for each sire as the total number of male offspring produced divided by the total number of female offspring produced. Therefore, ratios above 0.5 represent an excess of male offspring, whereas ratios below 0.5 represent an excess of female offspring. To determine if the sex ratio of the total sample was

significantly biased we compared the average sex ratio across sires to a sex ratio of 0.5 using a one sample *t*-test. To examine the relationship between brood mass weight and survival, we compared the average weight of brood masses yielding surviving offspring to those resulting in mortality across sires using a paired *t*-test. Likewise, we used a paired *t*-test across sires to compare the average weight of brood masses yielding male and female offspring.

To determine if females adjust offspring sex ratio with the quality of the provisioning environment, we analyzed our data from the experimental manipulation using a 3-dimensional chi-square analysis. Dung quality (horse or cow dung), brood mass weight manipulation (control or reduced) and offspring sex (male or female) were included as the categorical variables and the frequency of offspring in each cell was used as the response variable. To determine if male and female offspring have different resource requirements we performed a 3 factor ANOVA including dung type, brood mass weight manipulation, and the sex of the offspring as the main (fixed) effects, and the weight of the brood mass that offspring developed in as the dependent variable. The exclusion of non-significant interaction terms via model reduction did not alter our findings so we present the complete model.

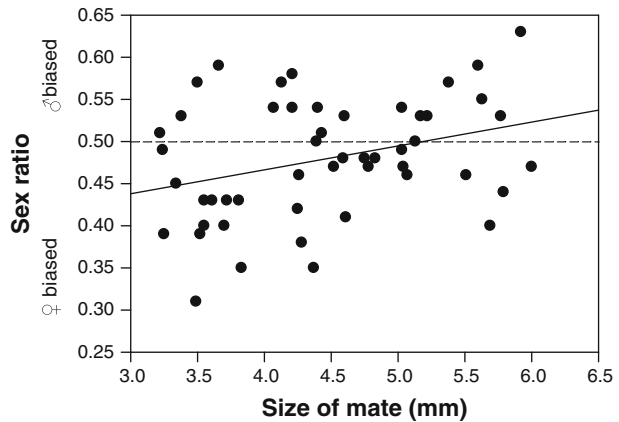
All statistical analyses were performed using procedures in the JMP® (version 7) statistical package and all means are presented ± 1 SE. For all of our statistical models we also calculated effect sizes to highlight the magnitude of our effects, following the standard protocols outlined in Cohen (1977) and in Nakagawa and Cuthill (2007). For all regression analyses, we calculated Cohen's f^2 and for all analyses involving *t*-tests we calculated Cohen's *d*. We calculated the effect size of each term in our chi-square analysis using Cramér's Phi (Φ) and in our factorial ANOVA using Cohen's \hat{f} .

Results

Sex ratio and mate attractiveness

Females mated to larger males produced significantly heavier brood masses ($\beta \pm \text{SE} = 0.11 \pm 0.05$, $r^2 = 0.10$, $t_{49} = 2.31$, $P = 0.025$, $f^2 = 0.11$) and more offspring ($\beta \pm \text{SE} = 3.13 \pm 0.83$, $r^2 = 0.23$, $t_{49} = 3.75$, $P = 0.0001$, $f^2 = 0.30$), thereby confirming our previous finding that females allocate more resources to reproduction when mated to large males (Kotiaho et al. 2003). Overall, we found a slight but significant sex ratio bias towards female offspring (one-sample *t*-test against a mean of 0.5; 0.48 ± 0.01 ; $t_{49} = 2.06$, $P = 0.045$, $d = 0.29$). However, the offspring sex ratio changed from being more strongly female biased towards being equal or even male biased as the size of the female's mate increased ($\beta = 0.03 \pm 0.01$, $r^2 = 0.11$, $t_{49} = 2.30$, $P = 0.026$, $f^2 = 0.12$) (Fig. 1). Offspring sex ratio was not biased by the average size of dams mated to a sire ($\beta = 0.11 \pm 0.20$, $r^2 = 0.006$, $t_{49} = 0.13$, $P = 0.90$, $f^2 = 0.006$) nor was it related to the average weight of the brood masses produced by females ($\beta = 0.03 \pm 0.04$, $r^2 = 0.02$, $t_{49} = 0.907$, $P = 0.37$, $f^2 = 0.02$). However, a paired analysis of brood masses produced by all females mated to the same male partner showed that offspring were more likely to survive to adulthood when they developed in heavier brood masses (dead = 3.22 ± 0.06 g; surviving = 3.43 ± 0.05 g; $t_{49} = 6.41$, $P = 0.0001$, $d = 0.35$). Among these surviving offspring, male offspring developed in significantly heavier brood masses (3.40 ± 0.04 g) than female offspring (3.30 ± 0.04 g) ($t_{49} = 8.96$, $P = 0.0001$, $d = 0.18$).

Fig. 1 The relationship between the size of a female's mate and the sex ratio of offspring. The solid line represents the regression line fitted through the data ($r^2 = 0.11$, $\beta = 0.03 \pm 0.01$) and the dashed line represents an equal sex ratio



Sex ratio and resource quality

We found a significant overall effect of dung quality, brood mass weight manipulation and offspring sex on the number of surviving offspring ($\chi^2 = 46.81$, $df = 4$, $P = 0.0001$, $\Phi = 0.195$). There was a significant effect of the brood mass weight manipulation on the number of surviving male and female offspring ($\chi^2 = 23.46$, $df = 1$, $P = 0.0001$, $\Phi = 0.138$), with an excess of females produced when brood mass weights were experimentally reduced but an equal sex ratio in control broods (Table 1). There was no effect of dung quality on the number of surviving male and female offspring ($\chi^2 = 0.57$, $df = 1$, $P = 0.450$, $\Phi = 0.021$) but significantly more offspring survived on horse (high quality) than cow dung (low quality), and more offspring were produced in the control than the reduction treatment (Table 1, $\chi^2 = 22.60$, $df = 1$, $P = 0.0001$, $\Phi = 0.135$). The overall interaction between the 3 factors was not significant ($\chi^2 = 0.17$, $df = 1$, $P = 0.68$, $\Phi = 0.012$).

There were significant effects of dung quality, brood mass weight manipulation and offspring sex on the weight of the brood mass produced by females (Table 2). As has been shown previously (Hunt and Simmons 2004), females produced heavier brood masses

Table 1 The number of male and female offspring surviving to adulthood in the control (unmanipulated) and brood mass reduction treatments when females provisioned brood masses with either horse (high quality) or cow (low quality) dung

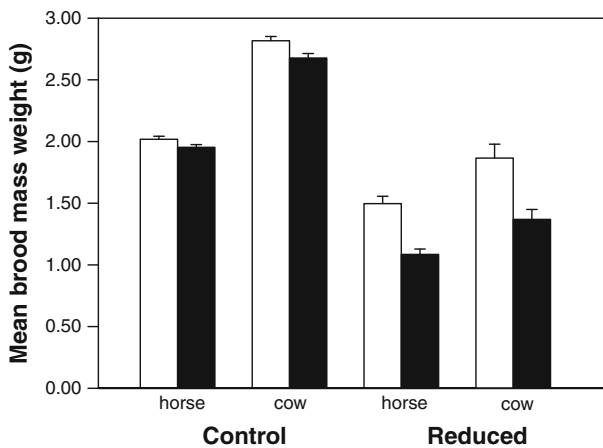
	Control		Reduced		Total
	Male	Female	Male	Female	
Horse	143	160	127	227	657
Cow	170	175	77	157	579
Total	313	335	204	384	1,236

As 400 brood masses were established at random in each of the four combinations of dung types and brood mass manipulations (control brood mass/horse dung, control brood mass/cow dung, reduced brood mass/horse dung and reduced brood mass/cow dung) prior to assigning sex to offspring, the bias in the number of male and female offspring surviving to emergence is due to sex-specific mortality. The number of offspring surviving in each of the four treatment combinations is simply the sum of the number of male and female offspring that successfully emerged. Subtracting this value from the 400 brood masses initially established gives the number of dead offspring in each treatment combination

Table 2 Three factor ANOVA examining how the weight of the brood mass provisioned by females varied with dung quality, brood mass manipulation and the sex of the developing offspring

Model term	<i>df</i>	<i>SS</i>	<i>F</i> -ratio	<i>P</i>	\hat{f}
Dung quality (A)	1	83.87	214.194	0.0001	0.415
Manipulation (B)	1	235.66	601.871	0.0001	0.697
A × B	1	13.38	34.164	0.0001	0.164
Offspring sex (C)	1	21.98	56.128	0.0001	0.212
A × C	1	0.46	1.163	0.2811	0.029
B × C	1	8.77	22.389	0.0001	0.130
A × B × C	1	0.002	0.005	0.9453	0.000
Error	1,228	480.82			

We also provide the Cohen's \hat{f} for each term in the model to assess effect size

**Fig. 2** The mean weight of brood masses producing male (white bars) and female (black bars) offspring in our brood mass weight (control or reduced) and dung quality (horse or cow) manipulations

when provisioning on cow dung (low quality) than on horse dung (high quality) and, not surprisingly, control brood masses were heavier than manipulated brood masses (Table 2; Fig. 2). Male offspring emerged from significantly heavier brood masses than female offspring (Table 2; Fig. 2). There was a significant interaction between dung quality and brood mass weight manipulation (Table 2) reflecting the larger reduction in weight when our manipulation was performed on brood masses provisioned with cow dung (low quality) compared to horse dung (high quality) (Fig. 2). There was also a significant interaction between brood mass weight manipulation and the sex of offspring (Table 2), reflecting the larger difference in the weight of brood masses producing male and female offspring in the brood mass reduction treatment versus the control treatment (Fig. 2). The interaction between dung quality and sex of offspring was not significant, nor was the overall interaction between all three main effects (Table 2).

Discussion

In theory, females should adjust their offspring sex ratio whenever the fitness gains of producing one sex is higher than the other (Trivers and Willard 1973). Here, we show that *O. taurus* exhibits a clear bias in offspring sex ratio and that this bias varies with the attractiveness of the female's mating partner, as well as with the quality of resources that a female has available for provisioning offspring. We found that an excess of daughters were produced when females mated to smaller, less attractive males and when larval provisions were experimentally reduced. While this finding raises the possibility of sex ratio manipulation by mothers, the complexity of this dung beetle system makes it difficult to predict exactly how selection is likely to act on sex ratio in this species. Clearly more empirical work is required, particularly with regard to how rapidly fitness increases with size in the sexes, before we can start to test explicit predictions of adaptive sex ratio theory in *O. taurus*. Nevertheless, our work provides a clear non-adaptive explanation for the sex ratio bias observed in this species. We found strong evidence for sex-specific offspring mortality, and that this was related to the amount of resources provided in the brood mass with sons being more likely to die when developing in smaller brood masses.

In our analysis of the Kotiaho et al. (2003) data set we found that the sex ratio shifts from female bias to an unbiased sex ratio as the size of the female's mating partner increased. However, rather than necessarily reflecting an adaptive allocation by females, we showed that this relationship could be explained by sex-specific differences in offspring mortality that is linked to the weight of the brood mass provisioned by females. Offspring survival increased with the weight of the brood mass and surviving male offspring developed in heavier brood masses than female offspring, suggesting that only females mating to a large partner are able to provide the nutritional resources required to support the development of male offspring. This effect was even clearer in our manipulative experiment where we showed a consistent deficit of male offspring across dung types when brood masses were reduced in weight. Furthermore, on both dung types male offspring emerged from heavier brood masses than female offspring, and this sex difference was far more pronounced in the brood mass reduction treatment. Collectively, our work shows that the observed sex ratio bias in *O. taurus* is caused by the higher mortality of male offspring and suggests that this might be linked to a higher demand for nutritional resources during development.

In general, the differential mortality of male offspring appears common in many species of birds and mammals (Clutton-Brock et al. 1985; Clutton-Brock and Iason 1986). While in some species this can occur through parents selectively killing (e.g. Burley 1986) or differentially provisioning (e.g. Clotfelter 1996) male offspring, there is a tendency for male mortality to be higher in species where males are the larger sex (Clutton-Brock et al. 1985; Kalmbach and Benito 2007). This suggests that selection for a large body size places higher energy demands on males during development. Consequently, at times of food shortage the larger sex may be more susceptible to starvation or disease, leading to increased mortality (Clutton-Brock et al. 1985; Clutton-Brock and Iason 1986). Although *O. taurus* is not sexually dimorphic in body size, there is evidence of male specific developmental costs. During early development, larvae accumulate resources that are subsequently allocated to the development of adult structures, of which some are predicted to be costly to produce (Nijhout and Emlen 1998). Specifically, empirical studies suggest that investment in immune function (Cotter et al. 2008), horns (Hunt and Simmons 1997), testes (Simmons and Emlen 2006) and genitalia (Moczek and Nijhout 2004; Parzer and Moczek 2008) draw considerable resources from a fixed pool of resources in this species.

Thus, the higher energetic demands and increased mortality of male offspring that we observe in *O. taurus* is consistent with differential developmental costs in this species.

The question of whether or not parents are able to adaptively adjust the sex ratio of offspring has received considerable empirical attention. Clear evidence of sex allocation has been shown in social insects where it has been argued that haplodiploid sex determination gives females more control over sex allocation (Ode and Hunter 2002). Evidence for sex allocation in vertebrates is more controversial (Cockburn et al. 2002; Cameron 2004; Ewen et al. 2004), yet there is some support that chromosomal sex determination has not constrained the evolution of sex allocation in these taxa (West and Sheldon 2002; Cameron 2004). Importantly, West and Sheldon (2002) showed in a meta-analysis across eight species of birds that females produced more sons when mating to more attractive males. The results we present here for *O. taurus* suggest that a similar sex ratio bias can arise through the sex-specific mortality of offspring. Consequently, although we are not the first to raise this issue (e.g. Cockburn et al. 2002; Ewen et al. 2004), our study provides direct evidence of the importance of considering differential offspring mortality in studies of sex ratio evolution. We urge caution when interpreting studies that have not taken offspring mortality into account. While taking account of mortality is likely to prove difficult, particularly in studies of wild populations, the increasing use of molecular genetic markers to determine offspring sex prior to substantial mortality (e.g. Ellegren et al. 1996) coupled with manipulative studies (e.g. Pryke and Griffith 2009), are likely to continue to provide important insight on the evolution of sex allocation.

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