Information constraints and the precision of adaptation: Sex ratio manipulation in wasps

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Sex allocation theory offers excellent opportunities for studying the precision of adaptation. One of the best-supported areas in the field of sex allocation is Hamilton's theory of local mate competition, which predicts female-biased offspring sex ratios when populations are structured such that mating takes place locally before females disperse. As predicted by local mate competition theory, females of numerous species, especially parasitoid wasps, have been shown to lay a less female-biased sex ratio as the number of females simultaneously laying eggs on a patch increases. It has usually been assumed that this sex ratio adjustment comes through individuals adjusting their behavior directly in response to the presence of other females. Here we show that in the parasitoid wasp *Nasonia vitripennis*, this shift in offspring sex ratios is primarily caused by the presence of eggs laid by other females and to a lesser extent by the presence of other females. We confirm that females are behaving as predicted by theory, but the way in which they do so is not as straightforward as is often assumed. Instead, even when there are multiple females on a patch, individuals still use the cues that are more commonly associated with sex ratio adjustment in response to sequential visits to a patch by females. This result provides a possible explanation for the observed variation in *N. vitripennis* sex ratios. More generally, it confirms the need to consider the mechanistic basis of a behavior to understand fully its adaptive value.

Sex allocation is one of the most productive and successful areas of evolutionary biology. Unified theory predicts that individuals should adjust the sex of their offspring in response to environmental conditions, and there is considerable empirical support for this prediction (1, 2). Furthermore, because the fitness consequences of sex allocation are often reasonably clear, a close fit can sometimes be expected between theory and data, allowing theory to be tested quantitatively as well as qualitatively. Consequently, a major outstanding problem is explaining the precision with which empirical data fits theoretical predictions (3, 4). This is also important more generally because sex allocation offers some of the clearest opportunities to test the precision of adaptation and the limits on the power of natural selection to produce perfect organisms (5, 6).

A possible major limitation on the ability of organisms to behave perfectly is the extent to which they can process relevant information about the environment (7, 8). We can expect an organism to follow theoretical predictions only if it can assess the underlying variables assumed by theory. Indeed, it can be shown that the quantitative and even qualitative predictions of theory depend on assumptions about how individuals process information about their environment (9, 10). Here, we examine limitations imposed by information processing on one of the best-understood examples of sex allocation: female-biased sex allocation in response to local mating in parasitoid wasps (11).

Hamilton's (12) theory of local mate competition (LMC) predicts that when mating takes place among the offspring of one or a few mothers, before only the daughters disperse, a female-biased sex ratio is favored. Empirical tests of this LMC theory have come predominantly from two different approaches, with females visiting patches either in groups simultaneously or alone, sequentially (ref. 11; S.A.W., D.M.S., and B. C. Sheldon, unpublished data). First, as the number of females simultaneously laying eggs on a patch increases, a less female-biased sex ratio is favored. Specifically, Hamilton showed that in haplodiploid species such as wasps, the evolutionary stable sex ratio s^* (proportion males) is given by $s = (N - 1)(2N - 1)/N(4N - 1)$, where N is the number of females, termed foundress number (14, 15). Second, if females visit a patch sequentially, then the second female should lay a less female-biased or even male-biased sex ratio, depending on the relative size of the brood laid by the two females. Specifically, Werren showed that $s^* = (\sqrt{[2r^*(T + 1)]} - 2r^*)/2T$, where r^* is the optimal sex ratio of the first female on the patch, s^* is the optimal sex ratio of the second female, and T is the relative brood size of the second female (16, 17).

There is considerable evidence to show that LMC can explain variation in the sex ratio across populations/species, as well as facultative adjustment of offspring sex ratios by individuals in response to local conditions (2, 11). In particular, there is support from a number of species for shifts of sex ratio in response to both the number of females laying eggs on a patch simultaneously and the relative brood size when females lay eggs on a patch sequentially (11). However, even in cases where there is a relatively close fit between theory and data, there is still much phenotypic and genetic variation that cannot be explained (3, 18, 19). Much of this variation could be explained by how females assess the relevant environmental variables (8).

Here, we are concerned with assessing the importance of different cues in shaping offspring sex ratios when multiple females are laying eggs on a patch simultaneously, as envisaged in the original Hamilton (12) scenario. It has generally been implicitly assumed that females directly assess N, the number of females on a patch (11). However, an alternative possibility is that females indirectly assess foundress number through cues such as detecting that another female has previously laid eggs on the patch. It is known that females use such cues when visiting a patch sequentially (11, 16, 20–23). Consequently, when multiple females are laying eggs on a patch simultaneously, the question becomes the relative use by individuals of these different cues: cues from the already parasitized hosts (the presence of eggs: "host" cues) and cues from the presence of other females ("social" cues) (24, 25). The use of indirect cues could provide a better rule of thumb if, in nature, different females tend to lay eggs sequentially on a patch rather than simultaneously.

We tested experimentally the relative importance of direct and indirect cues by using the parasitoid *Nasonia vitripennis*. The sex ratio behavior of this organism has been hugely influential, providing a classic textbook example of sex ratio adjustment in response to variable foundress number (26). We manipulated direct cues by varying the number of females laying eggs on the patch and indirect cues by using hosts in which eggs had previously been laid (parasitized) or not (unparasitized). We were able to untangle direct and indirect cues by using females

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Abbreviation: LMC, local mate competition.

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who could not lay eggs because we cut their ovipositors off. We followed the behavior of individuals by utilizing eye color mutants.

Methods

Study Organism. *N. vitripennis* (Hymenoptera: Pteromalidae) is a gregarious parasitoid of the pupae of numerous Diptera, including species of Calliphoridae and Sarcophagidae (27). Females oviposit up to 40 eggs in hosts, with male offspring emerging just before females (after ≈14 d at 25°C). Males have reduced wings and do not disperse away from the natal patch, instead mating with females as they emerge. Females, which are fully winged, disperse to find new patches of hosts after mating. More than one female may oviposit in a host (termed superparasitism), although females prefer unused hosts. The mating system fulfils the assumptions of simple models of LMC, and females alter their sex ratio behavior broadly in line with theoretical predictions (18). The wasps used in this experiment were from the WT strain HV6, derived from wasps collected from birds' nests during 2001 in Hoge Veluwe, The Netherlands, by L. Beukeboom. In addition, the red-eye mutant strain STDR was used to allow us to follow the sex ratio decisions of individual WT females. All wasps were cultured by using *Calliphora vomitoria* as hosts and maintained at 25°C under 16-h light/8-h dark conditions.

Experimental Design. The experiment consisted of independently manipulating the condition of the hosts in which a female could oviposit and also the social environment of the female while ovipositing. We generated two types of host: (*i*) unparasitized or (*ii*) parasitized during the 24 h before the experiment by a mated red-eye mutant female. We also generated three social environments: (*i*) the focal female alone; (*ii*) the focal female plus four red-eye mutant cofoundress females who could not oviposit (see below); and (*iii*) the focal female plus four red-eye mutant cofoundress females who could oviposit. There were therefore six treatment combinations allowing us to separate the information a female obtains about the number of females ovipositing on a patch: indirect information from the host (eggs already present or not) and direct information from the environment (other females present who may contribute eggs to the patch).

Mated 2-d-old HV6 females were collected at random from mass culture and individually given a 2-d pretreatment to facilitate egg development. Pretreatment consisted of 24 h with a single host in a 75 × 10-mm glass vial, which was then removed and discarded, followed by 24 h with honey solution. Red-eye mutant females were collected and pretreated in the same way as HV6 females. Pretreated HV6 females were then placed in 75 × 25-mm glass vials and randomly assigned to a treatment combination described above. Two hosts were provided for oviposition. After 1 h, one-way escape tubes were placed on the glass vials to allow females to disperse away from the patches, to limit unnatural superparasitism (11, 26). After 48 h, all females were removed and the two parasitized hosts incubated individually to identify brood to particular hosts and also to check that hosts were parasitized beforehand as expected. Upon emergence, all offspring were sexed, genotyped by eye color (as WT or red-eye mutant), and counted.

To prevent red-eye females from being able to oviposit, their ovipositors were ablated by using the following procedure. Between the first and second days of pretreatment, individual females were given a host, into which they quickly started to drill before oviposition. Using microdissecting scissors, the ovipositor was cut under the female's abdomen while the ovipositor was drilling down into the host. Females that escaped or managed to withdraw their ovipositor were discarded. Females survived the ablation procedure and subsequent experiment and behaved normally, exploring hosts during the experiment (D.M.S., unpublished observations). Because all ablated females were red-eye mutants, we were able to check the success of ablation by whether any red-eye offspring emerged from the experimental hosts. Because of the intrusive nature of the ablation experiment, we deliberately unbalanced the design to optimize statistical power and limit the number of destructive ablations (28). After checking hosts for appropriate red-eye mutant and WT parasitism, sample sizes for the treatment combinations ranged from 16 to 48.

Statistical Analysis. The sex ratio data were analyzed with generalized linear models with binomial errors and a logit link function, using S-PLUS 6 (Insightful, Seattle). A full model was fitted, including interactions, and then terms were deleted in a stepwise fashion to obtain the minimal adequate model as described in ref. 29. Significance was assessed by testing the change in deviance after the removal of a term from the model. Differences among treatment levels were also examined by model simplification (29). After fitting the full model, the data were then examined for overdispersion by dividing the residual deviance by the residual degrees of freedom to give the dispersion parameter; relatively large values of residual deviance suggest overdispersion and risk potential overestimation of the significance level (29). To account for this, the residual deviance was rescaled by the dispersion parameter and an F test was used to evaluate whether the removal of a term caused a significant increase in deviance. In addition, model fit was checked by examining the distribution of residuals. All other statistics were also carried out with S-PLUS 6.

Precision of Adaptation. We compared the sex ratios produced by females with different cues available to them to the sex ratios predicted by theory. Specifically, we compared the sex ratios produced by females on unparasitized hosts, with four cofoundresses who either could or could not oviposit, to the sex ratio predicted by Hamilton (14, 15) for haplodiploids. Because $n = 5$, the predicted sex ratio is 0.38. Sex ratios were arcsine-square-root-transformed and compared to the predicted sex ratio with a t test. We also calculated the fitness consequences for females of the observed sex ratios, calculating the expected fitness obtained through sons and daughters, following Hamilton (14, 15). We compared this to the maximal fitness of a hypothetical optimal female producing a sex ratio of 0.38. We also calculated the fitness of a hypothetical female who did not facultatively alter sex ratio, using the sex ratio of females in the treatment where focal females oviposited alone on unparasitized hosts. We repeated these calculations for the case when females visit a patch sequentially, i.e., the treatment where females visited parasitized hosts alone. The observed sex ratios of females were compared to predictions from the Werren superparasitism model adapted for haplodiploids (16, 30), which takes into account the relative clutch sizes of the females, using a paired t test (relative clutch sizes T ranged from 0.05 to 3.43, and predicted sex ratios ranged from 0.12 to 1 before transformation).

Results

Relative Importance of Direct and Indirect Cues. There was a highly significant effect of host pretreatment (previously parasitized or not) on sex ratio ($F_{1,176} = 59.43$, $P < 0.0001$) with females producing a less female-biased sex ratio in the hosts that were already parasitized (Fig. 1). There was also a highly significant effect of female social environment (alone, with females who are not ovipositing, or with females who are ovipositing) on the sex ratio ($F_{2,176} = 44.07$, $P < 0.0001$) with females laying a less female-biased sex ratio in response to the presence of other females and also if eggs are being laid by those females. There also was a significant interaction between host condition and social environment ($F_{2,174} = 4.82$, $P = 0.009$), with the overall full

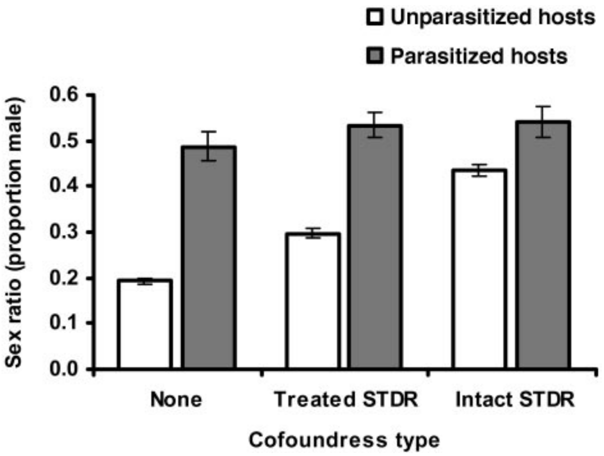

Fig. 1. Sex ratio in terms of host pretreatment (previously unparasitized or parasitized hosts) and social environment [alone, with treated red-eye mutant (STDR) females who cannot lay eggs, and with red-eye females who can lay eggs]. Error bars are binomial standard errors.

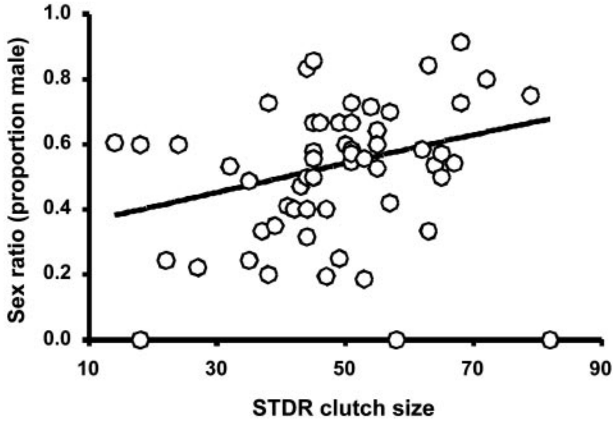

Fig. 2. Relationship between the sex ratios of females ovipositing on parasitized hosts and the overall clutch size that was laid previously on the patch by the red-eye mutants (STDR). Data from the three social environments are combined.

model explaining 46.7% of the variation in the data. This result is relatively strong, explaining ≈13 times the average amount of variation (3.6%) explained in evolutionary studies (31).

As Fig. 1 illustrates, the significant interaction is due to the negligible effect of social environment when hosts were already parasitized (shaded bars), compared with when the hosts were unparasitized (open bars). The differences between social environment treatment levels were all highly significant if the hosts were unparasitized (all $P < 0.0001$), whereas there was no significant difference among the three social environment treatments if the hosts were already parasitized ($F_{2,53} = 0.00$, $P = 0.99$). Therefore, on a previously unparasitized patch, females produced a less female-biased sex ratio when ovipositing with other females than when alone, and there was an effect even if those other females did not lay eggs. However, the biggest shift in sex ratio occurs if those cofoundress females do oviposit.

The relative importance of direct cues from the presence of other females and indirect cues from previously parasitized hosts can be most easily quantified if we consider the four treatment combinations where red-eye cofoundresses do not lay eggs (i.e., considering the effects of direct and indirect cues with a 2 × 2 factorial design). In this case, both host pretreatment and social environment are still highly significant ($F_{1,115} = 62.56$, $P < 0.0001$ and $F_{1,115} = 17.02$, $P < 0.0001$, respectively), but the amount of variance in sex ratio explained by host pretreatment ($R^2 = 31.5\%$) is much greater than that explained by social environment ($R^2 = 8.6\%$). Therefore, both social and host cues produce shifts in sex ratio, but the latter cues are the most important.

Clutch Size and Sex Ratio. When females superparasitize already parasitized hosts, they are expected to produce less female-biased sex ratios when superparasitizing larger broods (16, 30, 32). We checked for this effect and found there was a weak positive effect of STDR clutch size on sex ratio within the parasitized host treatment combinations ($F_{1,57} = 6.78$, $P = 0.012$) (Fig. 2). Females therefore did produce less female-biased sex ratios on hosts with more STDR brood. There was no significant difference in STDR clutch size between these treatment combinations (mean clutch size = 48.5 ± 1.9 eggs, one-way ANOVA: $F_{2,55} = 0.87$, $P = 0.43$). For the one treatment combination in which STDR eggs could have been present in previously unparasitized hosts (i.e., with intact STDR cofoundresses), there was also a significant positive effect of STDR clutch size ($F_{1,43} = 10.05$, $P = 0.003$). Again, these shifts in relation to the STDR clutch size illustrate the importance of host cues. Finally, there was no significant effect of HV6 clutch size on sex ratio in the experiment ($F_{1,173} = 1.79$, $P = 0.18$).

Precision of Adaptation. Females on a patch with four intact cofoundresses, who also laid eggs on the patch, produced a sex ratio not significantly different from that predicted by theory (predicted sex ratio = 0.38 and mean sex ratio = 0.43; $t_{45} = 1.47$, $P = 0.15$). In contrast, the sex ratio produced by females with four cofoundresses who were unable to lay eggs was significantly lower than that predicted (mean sex ratio = 0.30; $t_{27} = 3.63$, $P = 0.001$). The behavior of individuals therefore only agrees quantitatively with the prediction from theory if females are able to use both direct and indirect cues.

We then calculated the relative fitness of females with different cues. The mean fitness of females on a patch with intact cofoundresses was 99.7% that of an optimal female (giving a selection coefficient $s = 0.003$). The mean fitness of a female on a patch with treated females who could not lay eggs, and hence use only direct cues, was 99.4% that of an optimal female ($s = 0.006$). If we use the mean sex ratio of females who lay eggs on a patch alone (0.19) as the sex ratio in response to no cues, then these females have a fitness of 96.7% of the optimal female fitness ($s = 0.033$). The fitness differentials for different amounts of sex ratio change therefore appear to be small, but females clearly do still respond to both direct and indirect cues to increase the precision of their adaptive sex ratios.

We can also determine the fitness consequences of sex ratio adjustment when females visit a patch simultaneously, and hence only indirect cues can be used. The observed sex ratios of females laying eggs on previously parasitized hosts did not differ significantly from those predicted by the superparasitism model ($t_{24} = 1.21$, $P = 0.24$). The mean fitness of these females was 98.9% as fit as a hypothetical optimal female would be ($s = 0.011$). Again, using females who oviposited alone on unparasitized hosts as a reference, the sex ratio of females who did not facultatively alter their sex ratio was significantly different to the predicted sex ratios ($t_{24} = 5.42$, $P < 0.0001$), and these females would be only 94.3% as fit as an optimal female ($s = 0.057$).

Discussion

Females of many insect species have been shown to lay a less female-biased sex ratio if other females are simultaneously laying eggs on the same patch, as predicted by Hamilton's LMC theory (1, 2, 11, 12). We have shown that in the parasitoid wasp

N. vitripennis, this shift in offspring sex ratios is primarily caused by the presence of eggs laid by other females, and to a lesser extent by the presence of other females. Overall, these results suggest that females are behaving as predicted by theory, but the way in which they do so is not as previously envisaged for this kind of sex ratio adjustment. Instead, they are primarily using the same cues as when females visit a patch sequentially (11, 16, 17, 20).

Our study independently manipulated the different sources of information that a female may use to estimate the extent of LMC. Consequently, although our results confirm the importance of host condition and female cofoundresses on sex ratio (16, 20–26), they also highlight the subtlety of information use by *N. vitripennis* females. For example, if the hosts are already parasitized, the sex ratios produced are unaffected by the presence of other females. In this case, it is only the number of eggs from other females in the hosts that determines sex ratio (see Fig. 2). That females can in some way estimate the number of eggs in hosts is impressive, and the degree to which they can do this is another matter of importance (13, 18, 21, 22, 33, 34). If, on the other hand, the hosts were not initially parasitized, then the data show that the presence of other females does decrease the female bias in the resulting sex ratios and that this effect occurs even if those females do not lay eggs. However, the change in sex ratio is smaller than occurs if those females do oviposit, or if the hosts are already parasitized as discussed above (Fig. 1). Overall, our result clearly shows that the effect of a cofoundress on another female's sex ratio is mediated through the direct cue of the female being present, but more importantly by the indirect cues about previous oviposition gained from the host.

We estimated the extent to which different cues influenced the precision of sex ratio adjustment when females lay eggs on a patch simultaneously with other females. Females adjusted their sex ratio only in response to the number of females on the patch as predicted quantitatively by theory (12, 14, 15, 30) when they were able to use both direct and indirect cues. When only direct cues were available from the presence of other females who could not lay eggs, the sex ratio was significantly below that predicted by theory, reducing female fitness, although not by as much as if they had made no response at all.

Our results have two implications for understanding sex ratio adjustment in response to LMC. First, previous work has emphasized the distinction between sex ratio adjustment in response to the number of females on a patch (Hamilton's classic LMC model) and that in response to eggs having already been laid on a patch (Werren's superparasitism model) (34). Our results show that this distinction is artificial at the mechanistic and behavioral levels. Furthermore, this finding unites the empirical data with recent theoretical overviews. From a theoretical perspective, extremely general LMC models can be constructed, which can then be applied to simultaneous or sequential oviposition, emphasizing that the same fundamental selective factors are at work in different situations (30, 35, 36).

Second, our results can help explain some of the variation in sex ratio behavior that is often observed between individuals (24, 37). If the presence of eggs laid by other females is the major cue determining offspring sex ratios, then individuals would be expected to adjust their behavior over time on a patch, adding extra noise into the system. This behavior also will depend on the relative rate at which different females lay eggs, which can vary with factors such as body size and past experience (38, 39). At the very least, more cues can lead to more variation. Furthermore, it suggests that the presence of eggs can be a more reliable/useful cue for the extent of LMC than the simultaneous presence of other females. This would be expected if females tended to visit patches sequentially in nature or if females visiting a patch simultaneously tended to lay very different clutch sizes (30, 32, 34, 40, 41). Consequently, the classic experimental protocol of varying the number of females on a patch (N) may be somewhat artificial for some species and could explain some of the variation across species in the extent to which individuals shift sex ratio with foundress number (3). In particular, it could lead to females overestimating the extent of LMC (underestimating the foundress number) in laboratory experiments. Furthermore, it suggests that differences across species in the cues used to adjust offspring sex ratios (2, 11, 26, 42) may reflect biological differences in the distribution of hosts and ovipositing females, which in turn determine the most useful cues (rules of thumb) for each species.

Conclusion

Our results and other recent work on sex allocation demonstrate how an understanding of mechanistic issues can be crucial to understanding adaptation. Other examples include: (*i*) social insects, where the mechanism by which conflict is resolved between workers and queens can determine the quantitative and even qualitative predictions made by evolutionary models (43–45, 48); and (*ii*) protozoan parasites, where the extent of sex ratio adjustment depends on the extent to which the parasites can assess factors such as parasite genetic diversity and host immune response (46, 47). Work on sex allocation provides clear quantitative examples in that in some cases a full understanding of the adaptive nature of a trait ("why" questions) can be obtained only with an understanding of the underlying mechanisms ("how" questions).

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