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Evolutionary Biology: Parasite, Know Thyself

Studies of sex allocation provide some of the best evidence for Darwinian adaptation in nature. A new study of malaria parasites provides striking support for this cornerstone of evolutionary biology, with important implications for both evolutionary and medical biology.

Sarah C.L. Knowles
and Ben C. Sheldon

Sex allocation theory attempts to explain how natural selection shapes the way organisms divide resources between male and female offspring. What is often referred to as ‘fisherian’ sex allocation, after the explanation in R.A. Fisher’s key text [1], is the existence of an evolutionarily stable sex allocation where resources are equally divided into males and females. This applies only for the special case where there is random mating, and no inbreeding, as was first pointed out by W.D. Hamilton in 1967 [2]. Hamilton realised that, as mating became increasingly less random, natural selection would favour sex ratios skewed increasingly more strongly towards females, because this would reduce wastage of resources on males that compete with each other for the same fertilisations. Life cycles that favour this type of sex allocation behaviour are quite widespread, and while much of the work in this field has been on arthropods, it was pointed out some time ago by Read *et al.* [3] that the life cycle of malaria parasites selects

for the evolution of ‘hamiltonian’ sex ratio behaviour. A new study by Reece *et al.* [4], using elegant experimental and molecular techniques, has now provided the first conclusive evidence that several key assumptions and predictions of sex allocation theory are upheld in malaria parasites: these parasites appear to show considerable sophistication in their ability to adjust sex ratio in response to the prevailing conditions within their host. The findings also have some fascinating implications for our understanding of the fundamental biology and within-host behaviour of malaria parasites.

Hamilton’s sex ratio theory is often illustrated using the example of fig-pollinating and parasitoid wasps (Figure 1A), where newly emerged females are commonly confined to a patch in which the only potential mates are their brothers. The resulting ‘local mate competition’ leads to the optimal offspring sex ratio being very strongly female-biased: a mother’s fitness is maximized if she produces just enough sons to mate with all her daughters. At the other extreme, in a fully outbred (fisherian) population the optimal strategy is to produce equal

numbers of sons and daughters. Hamilton showed that, under local mate competition, the unbeatable sex allocation strategy (proportion of males produced, r^*) depends on the inbreeding rate according to the equation $r^* = (1-f)/2$, where f is Wright’s coefficient of inbreeding. An extension of this theory would predict that, if the level of inbreeding offspring experience is variable, natural selection favours a facultative sex allocation strategy, in which females adjust their offspring sex ratio to maximize the production of grandchildren; such facultative sex allocation has been observed in a wide range of taxa [5,6].

Just like fig-pollinating wasps, malaria parasites (Figure 1B) experience variable levels of inbreeding as a result of mating within a small ‘patch’ shortly after reaching sexual maturity. Sex in malaria (*Plasmodium*) and related Apicomplexan parasites (such as *Haemoproteus* and *Leucocytozoon*) occurs when sexual stages called gametocytes that circulate within the vertebrate bloodstream are taken up by a vector when it takes a blood meal. Mating occurs inside the vector gut, when sperm-like gametes are released from male gametocytes to seek out and fuse with the larger female gametes. Since most malaria infections are made up of only a few of the many genotypes present in the whole population, malaria parasites generally experience some degree of inbreeding. In accordance with local mate competition theory, their sex ratios are

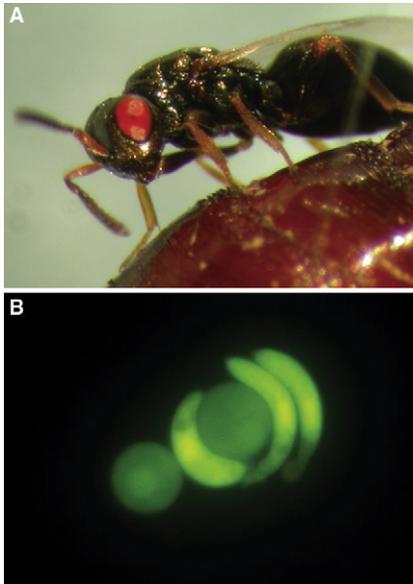


Figure 1. Adaptive sex-ratio adjustment. (A) Parasitoid wasps, such as *Nasonia vitripennis* (the photo shows a red-eye mutant to facilitate fitness measures in experiments) have long been known to adjust their sex ratios to the likelihood of inbreeding. (B) Malaria parasites (the photo shows *Plasmodium berghei* expressing a green fluorescent protein) have now been shown to perform the same behaviour. Fertilised female parasites (elongate and banana shaped) can easily be distinguished from unfertilised female gametes (spherical), allowing the reproductive success of mating groups to be measured. In both cases, the fact that mating takes place between relatives is the key to understanding the behaviour. Photos: (A) Stuart West; (B) Sinclair Stammers.

consistently female-biased, and the degree of female-bias is often, though not always [7], correlated with factors that might predict the degree of inbreeding across populations [8,9]. Despite this suggestive evidence, elucidating the true role of local mate competition in shaping malaria parasite sex ratios has proven rather difficult. All data to date have been observational rather than experimental, and thus cannot address whether malaria parasite sex ratios are adapted to the average rate of inbreeding experienced, or whether they are adjusted facultatively, depending on the parasite genetic diversity within a given infection. To complicate matters further, it is known that gametocyte sex ratios can be highly variable within single malaria infections across time, and that additional factors, such as host anaemia, can cause malaria parasites to shift their sex ratio [10,11].

Reece *et al.* [4] overcame several of these issues by conducting carefully controlled experiments using the rodent malaria parasites *P. chabaudi* and *P. berghei*. They first used a cunning genetic-modification technique, in which genes for either male or female gamete fertility had been knocked out, to carry out an experimental test of the effect of gametocyte sex ratio on fitness. By mixing different proportions of these two knock-out strains, they could construct mating groups with experimentally-determined sex ratios and study the effect of these sex ratios on the production of ookinetes (zygotes) *in vitro*. These experiments demonstrated clearly that the sex ratio is under stabilizing selection — that is, that selection acts strongly against very extreme sex ratios — under these conditions. While this may not sound particularly dramatic, it is — remarkably — the first published, fully controlled experimental test of the effect of sex ratio on fitness in any system, and has potentially important implications for understanding transmission of the parasites.

Second, by inoculating naïve laboratory mice with either single or multiple genotypes of *P. chabaudi*, and monitoring the gametocyte sex ratio over time, the authors were able to demonstrate that malaria parasites do indeed alter their sex allocation strategy according to the level of inbreeding. Overall gametocyte sex ratio was significantly less female-biased within infections produced by simultaneous inoculation with six parasite genotypes as compared with single genotype infections. Moreover, using newly developed quantitative sex-specific genetic techniques, Reece *et al.* [4] investigated whether individual genotypes could facultatively adjust gametocyte sex ratio when in the presence of an unrelated genotype. Two of the three genotypes investigated in depth did produce a less female-biased sex ratio when an unrelated genotype was present, just as local mate competition theory would predict. The mechanistic implication underlying this discovery is that malaria parasites are somehow able to detect the presence of genetically distinct genotypes within their vertebrate host: in other words they are capable of kin discrimination. Furthermore, the authors report a negative correlation

between the gametocyte sex ratio of focal genotypes and their proportional representation in the mating group. What is striking is that this relationship is in close quantitative agreement with the predictions of local mate competition theory and suggests the intriguing possibility that malaria parasites can not only detect presence or absence of unrelated genotypes, but can even assess, by some means, their own relative abundance within an infection. The support for this theory was not unanimous, as not all clones showed the ‘appropriate’ behaviour, and there were large, currently unexplained, differences in mean sex ratios among clones under the same environment. Understanding variation in sex ratio adaptation remains an important challenge for the field as a whole [12].

While the experiments of Reece *et al.* [4] provide striking support for the assumptions and predictions of sex allocation theory, their implications for understanding malaria in a biomedical context are perhaps equally, if not more, important. In a similar vein to local mate competition theory, kin-selection models of virulence predict that if the genetic composition of infections is variable, selection will favour parasites able to adjust their virulence in response to the presence of co-infecting genotypes, with mixed genotype infections resulting in higher overall virulence than single infections [13,14]. If parasites can detect non-kin and adjust their gametocyte sex ratio accordingly, it is entirely plausible they could adjust their host exploitation strategy in a similar way. Reece *et al.*'s [4] success in applying evolutionary theory to the sex ratios of malaria parasites encourage the idea that a similar approach could shed light on the fundamental relationship between multiplicity of infection and pathogenicity in malaria.

If, as the data strongly imply, malaria parasites can discriminate kin within infections and alter their behaviour in response, this is a major advance in our understanding of their basic biology, which has the potential to shed light on various other mysteries of malariology. For instance, the phenomenon of antigenic variation, whereby an entire population of parasites switches its surface antigen to avoid immune recognition is a major obstacle to the development of a malaria vaccine. Yet

we still do not understand exactly how the population-wide co-ordination of this switch is achieved [15,16]. If malaria parasites really can detect kin and respond to their presence, their means of within-host communication are much more sophisticated than hitherto realised, and exploring the underlying mechanisms could prove hugely enlightening.

The experimental demonstration that gametocyte sex ratio influences ookinete production *in vitro* is an important milestone, and suggests that studies assessing the determinants of transmission success in malaria should take account of gametocyte sex ratio in addition to other factors such as gametocyte density and maturity. Reece *et al.*'s [4] contribution to the body of work showing that gametocyte sex ratio is a flexible fitness-determining trait that can be adjusted in response to a variety of factors also re-emphasizes the importance of bearing in mind such crafty parasite tricks when designing interventions like anti-malarial drugs and transmission-blocking strategies.

In conclusion, this recent work [4], apart from providing strong and novel support for sex allocation theory demonstrates yet another way in which

this medically important parasite displays a form of sophisticated and responsive behaviour. That the responses hinge on the apparent ability of the parasites to discriminate kin is exciting, and suggests a form of social behaviour in these organisms that might not be generally expected. It also raises question of how studies of, and interventions against, this deadly parasite should take account of its flexible adaptive behaviour.

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Synaesthesia: The Sounds of Moving Patterns

A newly reported form of synaesthesia in which seeing visual motion induces auditory experiences challenges traditional ideas about the neural mechanisms of synaesthesia and may shed light on how the brain integrates information from sound and vision.

Edward M. Hubbard

In synaesthesia, sensory and cognitive experiences lead to additional, unusual experiences, such as seeing colours when looking at letters or numbers [1] or when listening to speech [2], or even tasting flavours in the mouth in response to musical intervals [3]. Although synaesthesia was first brought to the attention of the scientific community over 100 years ago, the neural mechanisms that lead to these experiences are still debated. In this issue of *Current Biology*, Saenz and Koch [4] report

a previously unknown form of synaesthesia — ‘hearing-motion’ synaesthesia, in which seeing moving or flickering visual patterns leads to specific auditory experiences. This form of synaesthesia raises numerous questions about the neural basis of synaesthesia, and promises to shed light on fundamental questions about how the brain integrates information from multiple sensory modalities.

In order to demonstrate the reality of these synaesthetic experiences, Saenz and Koch [4] asked subjects to identify whether two successively presented auditory (beeps) or visual (flashes)

sequences were the same or different. Consistent with previous studies, the authors showed that non-synaesthetic control participants were more accurate with auditory sequences than with visual sequences. For each of four hearing-motion synaesthetes, however, performance was no worse for visual sequences than for auditory sequences, consistent with their reports of hearing sounds in response to the visual flashes. This perceptual advantage provides an objective demonstration of the reality of the synaesthetes’ reports.

This form of synaesthesia is a challenge for traditional accounts of the neural basis of such experiences, which suggest that synaesthesia arises either from cross-activation between adjacent cortical regions [1] or reduced inhibition of feedback from multisensory areas [5]. Although many visual areas respond strongly to simple flashes and motion, none of these lie