

# Water-flea males from the netherworld

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**Simple treatments with hormones could unlock the expression of complex phenotypes not known to occur in nature. Using this method, Kim *et al.* recently obtained males from all-female populations of water fleas. The novel characters revealed by this work can be used in taxonomic identification and phylogenetic inference. Additionally, these ‘resurrected’ males offer insights into the conservation of traits that are not exposed to natural selection.**

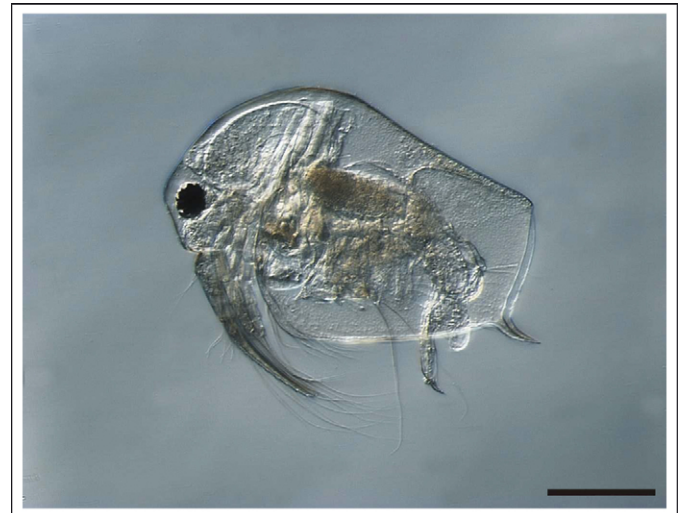
## Hormone-induced males

Methyl farnesoate (MF) is a crustacean hormone known to induce male formation in the water flea *Daphnia magna* (Crustacea; Cladocera) [1]. This species exhibits cyclical parthenogenesis, that is asexually reproducing all-female generations alternate seasonally with sexually reproducing generations that include males and females. Sex is determined environmentally in the Cladocera [2], and MF is thought to be the endocrine factor that ‘translates’ environmental cues into a developmental switch from the production of female offspring to one of males, thus initiating the sexual reproductive phase [1].

In a recent paper, Kim *et al.* [3] demonstrate that MF elicits the same male-inducing effect in other lacustrine cladocerans, including three species where males are not known to occur in nature (Figure 1). In cladocerans, as in many other animal groups, male morphology provides better taxonomic characters than does female morphology, for example, in the shape of the abdomen and the sensory structures of the antennules. Therefore, the males hormonally obtained by Kim *et al.* provide precious, otherwise unavailable characters of diagnostic value in the taxonomy of the originally all-female populations and are potentially informative for reconstructing the phylogeny of the group.

## Revealing taxonomic cryptic traits

Cryptic genetic variation is defined as standing genetic variation that does not contribute to the range of phenotypes normally observed in a population, but that is available to be expressed in novel phenotypes that result from altered environmental or genetic conditions [4]. Exposing organisms to physicochemical conditions that induce the expression of phenotypic features not usually seen under ordinary environmental circumstances is a clever way to uncover cryptic variation, both within and between species. A recent example of taxonomic information obtained in this way comes from a group of marine crustaceans (Facetotecta), in which a first post-metamorphic stage was



**Figure 1.** Hormonally induced male of the water flea *Bosmina liederii*. Scale bar = 0.1 mm. Image reproduced courtesy of Derek J. Taylor and Keonho Kim.

revealed as a result of chemical induction; previously, these crustaceans were known only from planktonic larval forms.\*

In molecular phylogenetics and taxonomy, relationships are reconstructed on the basis of the molecular composition of organisms irrespective of their morphological appearance. By contrast, although with the same aim of relationship reconstruction, hormonal manipulations amplify naturally occurring morphological diversity.

If the artificially induced males produced by Kim *et al.* are to be used systematically for phylogenetic analysis, it will be interesting to see whether the results are affected by problems such so-called ‘long branch attraction’. This is a common bias in phylogenetic inference, owing to rapidly evolving lineages tending to appear to be closely related, regardless of their true relationships [5–7].

If the taxon sample includes obligate parthenogens (i.e. populations that reproduce exclusively asexually), rapid evolution of characters related to the mate recognition system in hormonally induced males could result from genetic drift owing to the interrupted coevolution of male and female phenotypes. This adds to the divergence caused by the higher rate at which mutations tend to accumulate in asexual lineages, compared with their sexual relatives. In asexual lineages, genes are permanently linked owing to the lack of recombination. Thus, because natural selection cannot act independently on individual loci, the efficiency

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\* H. Glenner *et al.* (2005) Metamorphosis in Y-larvae (Facetotecta): towards the solution to a 100 year old riddle. Sixth International Crustacean Congress, Glasgow, 18–22 July, 2005, Book of Abstracts, 4.

of 'purifying' selection is reduced, favouring the accumulation of slightly deleterious mutations [8].

### Survival of traits invisible to selection

Species where males have never been observed are not necessarily all-female. Males can be cryptic, rare or produced only under exceptional conditions. For example, males have been found in a newly described species of living darwinulids, a group of ostracods (Crustacea) long believed to have been asexual since the Late Triassic, some 200 million years ago [9]. However, the presence of rare males does not necessarily imply that sexual recombination is occurring, as these males might be nonfunctional. Thus, clonality is best tested for by using population genetics tools [10].

There is no molecular evidence available for the occurrence of obligate parthenogenesis in the apparently all-female species discussed by Kim *et al.* [3]. Because of their rarity and possible non-functionality, even if males of these species do occur naturally, it would have little effect on the evolutionary implications of male phenotype conservation. Population genetic models show that rare sexual reproduction can be disadvantageous, thus, sexuality in such populations tends to disappear, leading to obligate asexuality [11].

### Broad-ranging implications for evolutionary biology

As noted by Kim *et al.* [3], their results suggest that the role of MF as final determinant of male formation is conserved among cladocerans. This is in agreement with a general model for the evolution of the gene regulatory cascade for sex determination in animals, where genes at the bottom of the cascade are more conserved than those at the top [12]. But how much of the morphogenetic processes downstream of this sex-determining event are also conserved? In terms of exposure to sexual (and non-sexual) natural selection, the male traits that are uncovered by the hormonal treatment are not equivalent to those of males in lineages that are cyclically parthenogenetic.

Naturally occurring functional males are not only exposed to non-sexual selection, but also interact with conspecific females, thus maintaining and evolving sexually selected traits [13]; this is not true of males induced by any means in asexual lineages. Here, the male phenotype is simply an alternative phenotype of the genome of the female, a kind of 'luxury example' of phenotypic plasticity. In principle, this description in terms of phenotypic plasticity would also apply to the seasonally occurring males of cyclical parthenogens, although, in this case, the periodic male–female interactions ensure the coevolution of male and female phenotypes.

Thus, the males resurrected from the netherworld by hormonal treatment are neither the males of past sexual generations of what are now (possibly) all-female populations, nor the undisclosed companions of extant females. We do not yet have a category that could incorporate naturally unexpressed phenotypes of asexual lineages.

### Evolutionary inertia of developmental processes

Kim *et al.*'s study [3] suggests that there is evolutionary inertia of the developmental trajectories leading to male

formation in these cladocerans, despite the lack of continuing selection on the corresponding phenotypes. If this is the case then it is another addition to the list of experimentally induced phenotypes that demonstrate the conservation of phenotypic potential that is no longer expressed in nature. An example is the conserved ability of the oral epithelium of the chicken embryo to interact with the neural crest-derived mesenchyme to form teeth, despite the fact that birds lost their teeth some 70–80 million years ago [14]. The loss of functional males in cladocerans is, however, not as old as the loss of teeth in birds: for several Northern water flea populations, this loss might date back to the late Pleistocene (120 000–12 000 years ago) [15].

However, for examples of complex and costly phenotypes that are maintained despite the lack of continuing selection, we do not need experimental manipulations. In our less-than-perfect world, we should not expect selection to be so efficient as to delete immediately unused traits or entire phenotypes. An example of a surviving relic phenotype are the adults of *Micromalthus debilis*, a beetle whose life history differs substantially from the standard life cycle of all other beetles. In this species, reproduction is by paedogenesis, that is, eggs mature in the body of the larva. Although the larva can metamorphose to become a winged adult under specific (but not yet fully known) conditions, it has not yet been demonstrated that the male or female adults have any part in reproduction [16]. If this is the case, then this is an example of complex metamorphosis being retained without an obvious advantage to the insect. One might wonder how much the morphology of these 'virtual adults', which has been regarded by taxonomists as peculiar enough to justify placing *M. debilis* in a family of its own, might reflect a drift associated with the putative lack of selection on this post-reproductive stage.

### Conclusion

Kim *et al.*'s results [3], and the stories of sex, reproduction and evolution that they evoke, should stimulate evolutionary biologists to relax, time and again, categories such as 'male' and 'female', 'sexual' and 'asexual', traditionally adopted as frames for the questions that we address. In particular, asexual and sexual reproduction should be recognized as two extremes of a continuum of reproductive modes [17]. Such an appreciation will aid our understanding of paths of evolutionary change that often do not care for our cherished logics.

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# Cooperation should not be assumed

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**Evolutionary game theory provides a framework for explaining social interactions, including those between males and females. In a recent article, Roughgarden *et al.* discuss a new approach to sexual selection based on cooperative game theory and argue that cooperation rather than competition is fundamental in interactions between the sexes. However, compelling reasons for adopting this approach are not given and the authors do not adopt it consistently. We argue that non-cooperative game theory provides an adequate basis for understanding sexual selection, but that further work is needed to produce realistic models. We agree with Roughgarden and colleagues that bargaining is an important aspect of social interactions, but this is not a novel claim. Bargaining does not require the assumption of cooperation and does not necessarily lead to it.**

## Introduction

The current approach to explaining sexual selection is based on the idea that individuals act in their own best interests. This often leads to a conflict between males and females [1]. In a recent paper that criticizes this idea [2], Roughgarden and colleagues argue for a new approach in which non-cooperative game theory (i.e. competition) is replaced by cooperative game theory (i.e. cooperation). Many authors have discussed various aspects of the claims of Roughgarden *et al.* in the Letters section of *Science* for May 5 2006 (Vol. 312, pp 689–697). Here, we concentrate on the challenge to evolutionary game theory.

## Evolutionary game theory

Evolutionary game theory provides a framework for understanding how evolution shapes interactions between organisms (i.e. it forms the basis for explaining social

interactions). The standard use of evolutionary game theory assumes that individuals act so as to maximise their own fitness. Each member of a population has a genetically determined strategy that specifies how it interacts with other population members. The strategy adopted by most population members at evolutionary stability is called an evolutionarily stable strategy (ESS) [3]. Under this strategy, each population member is using the best strategy given that others are also using this strategy. Thus, at an ESS, no individual can increase its fitness by adopting a different strategy. This is the definition of a Nash equilibrium (NE) (which Roughgarden *et al.* [2] call a ‘Nash competitive equilibrium’ or NCE). Cooperation is not assumed, but can emerge from each individual trying ‘selfishly’ to maximise its own fitness.

Many biological interactions between two organisms are modelled as a game in which each player makes a single decision in the absence of information about the decision of the other player. We refer to this as a one-shot simultaneous game. In such games, it is usual to assume that the choice of action of an animal is not influenced by the behaviour of its opponent, but is fixed, being determined by the genotype of the animal alone. Roughgarden *et al.* [2] are right to point out that one-shot simultaneous games are often unrealistic and we should take account of interactions between the players during the course of the game; however, this point has been made before in the context of interactions between males and females [4], and various models include interactions between animals [4–11]. When bargaining occurs, it is the rules (i.e. strategies) that govern bargaining that are genetically determined (and hence subject to evolutionary processes). At an ESS, rules are best responses to each other and are hence in NE [4]. Biological models in which rules evolve sometimes predict more cooperation than does the NE based on one-shot games, sometimes less [4,7,8].

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