

The bitterling–mussel interaction as a test case for co-evolution

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The European bitterling *Rhodeus sericeus* (Cyprinidae) spawns in the gills of freshwater mussels (Unionidae) and shows some obvious adaptations to this type of spawning, such as the development of an ovipositor. Furthermore, recent studies have shown that the fish avoid species of mussels that have a high likelihood of ejecting their eggs prematurely. This leads to the question of whether the interaction between bitterling and mussels could represent a case of co-evolution, involving evolutionary responses by both species to selection imposed by the other. The evidence for and against co-evolution is reviewed, incorporating new results from two sets of experiments designed to test for adaptive choices by bitterling according to the mussels' sex and reproductive state, as well as a preliminary study of potential benefits for mussels from exposure to bitterling. Host preferences by bitterling, both among and within mussel species, may indeed have evolved in response to differences in benefits for offspring survival. There is no evidence yet for any benefits to mussels from receiving eggs, whereas there are costs due to reduced ventilation rates when the gills contain bitterling eggs. While there are differences among mussel species and individuals in their tendency to reject bitterling embryos, these differences do not provide strong evidence for co-evolution. For example, they may reflect differences in host physiology such as ventilation rate and generalized responses to expelling objects from their gills. Therefore, while bitterling are well adapted for their obligate spawning relationship with mussels, it has been much more difficult to find evidence for adaptations by mussels for dealing with bitterling. This suggests that any co-evolutionary dynamics between bitterling and mussels may be asymmetric, with stronger responses to selection by the fish than by mussels.

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INTRODUCTION

Co-evolution involves a series of reciprocal evolutionary changes in two or more non-interbreeding populations that have a close ecological relationship and act as agents of natural selection on each other (Thompson, 1994). Theoretical advances in the understanding of co-evolution and adaptive traits have come from investigations of a range of host–parasite interactions (Anderson & May,

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1982; Thompson & Burdon, 1992; Takasu *et al.*, 1993; Takasu, 1998), including detailed behavioural studies of avian brood parasitism (Rothstein & Robinson, 1998; Davies, 2000).

A mutual congruence of traits between a pair of species does not necessarily indicate co-evolution. For example, each trait may have evolved independently in response to other forms of selection, and only those pairs of species that carry complementary traits may be able to interact with one another. Furthermore, it is possible for only one species to evolve adaptations to the other. In the case of parasitism this could occur if costs of defence outweigh benefits (the ‘equilibrium hypothesis’) or if the parasitic species has been able to evolve more quickly than the host (the ‘evolutionary lag hypothesis’) (Thompson, 1994). The most convincing demonstrations of co-evolution require measurements of costs and benefits to each participant, as well as evidence of evolutionary responses by each participant to selection imposed by the other.

This paper aims to review the evidence for co-evolution between a species of fish, the European bitterling *Rhodeus sericeus* (Pallas) and freshwater mussels (Unionidae) that serve as foster parents for its young. First, general adaptations by bitterling to spawning in mussels are reviewed, as well as findings from recent studies that indicate adaptive host preferences by bitterling both in relation to particular mussel species and the prior presence of bitterling embryos within species. New data are then provided from experiments that test for adaptive preferences by bitterling according to individual mussels’ sex and reproductive state. The relationship is then examined from the mussels’ perspective. This includes evidence that bitterling are costly to their hosts, as well as new data which do not support the idea that mussels might benefit from bitterling sperm. This suggests that the relationship is parasitic rather than mutualistic. These results are used to consider the evidence for co-evolution, and the applicability of the evolutionary lag and equilibrium hypotheses for explaining the relationship.

GENERAL ADAPTATIONS OF BITTERLING TO MUSSELS

The European bitterling, a Cyprinid, is a member of the subfamily Acheilognathinae. As with other members of the subfamily, it has an obligate spawning relationship with living freshwater mussels. It co-occurs with four main species of freshwater mussels over much of its European range: *Unio pictorum*, *Unio tumidus*, *Anodonta anatina* and *Anodonta cygnea* (Reynolds *et al.*, 1997; Smith *et al.*, 2000a; Mills & Reynolds, 2002a). During the reproductive season male bitterling defend territories around one or more mussels and attract females with courtship displays (Candolin & Reynolds, 2001; Mills & Reynolds, 2003). Males are highly aggressive towards other males, especially after females spawn (Candolin & Reynolds, 2002a). Female bitterling inspect the available mussels and insert their long ovipositors through the exhalant siphon of mussels and lodge the eggs in the gills of the host (Wiepkema, 1961; Heschl, 1989). The eggs are fertilized when the male bitterling’s sperm is drawn into the mussel with the inhalant respiratory current (Duyvene de Wit, 1966). The eggs develop within

the mussel for a period of 2–4 weeks, until the yolk sac is absorbed, whereupon the young bitterling leave *via* the mussel's exhalant siphon (Reynolds *et al.*, 1997; Reynolds & Guillaume, 1998; Aldridge, 1999a).

Members of the bitterling subfamily have several obvious adaptations for spawning in mussels. The ovipositor of the female, which grows to over half of the females' body length, is extremely rare among fish species. Within the Cyprinidae, the smaller ovipositor of the Dalmatian barbelgudgeon *Aulopyge huegelii* (Heckel) has probably evolved independently. This species deposits eggs into fissures within the substratum (Freyhof, 1997). The genus *Sarcocheilithys* (Gobioninae) also contains species that have ovipositors, and spawn in unionid mussels. The phylogenetic position of these Asian fishes is uncertain (Cunha *et al.*, 2002).

Bitterling have evolved further physiological adaptations for spawning in mussels. Females produce a smaller number of eggs that are much larger than those of most other species in the family (*e.g.* 300–400 eggs per season, measuring 2.6×1.7 mm; Aldridge, 1999a; Smith *et al.*, 2000b). For example, the common carp *Cyprinus carpio* L. produces 40 000 to 2 000 000 spherical eggs measuring 1.25 mm (Maitland & Campbell, 1992). The large size of bitterling eggs fits with predictions from the 'safe harbour hypothesis' (Shine, 1978) whereby selection should favour large egg size when eggs are exposed to low predation, as would be expected in mussels in comparison with rates of predation faced by eggs that are scattered on vegetation, for example. This is because eggs can afford the longer developmental time associated with larger size. Presumably, low fecundity has evolved due to the tradeoff with larger egg size (Smith & Fretwell, 1974).

Bitterling embryos may also be adapted to their hosts in response to conditions during development. The embryos compete with their mussel hosts for oxygen (Smith *et al.*, 2001), and may therefore experience low oxygen conditions. Furthermore, bivalves close their valves in response to environmental stressors (Kramer *et al.*, 1989; Reynolds & Guillaume, 1998) or adverse conditions (Storey & Storey, 1990) and are able to tolerate the anoxic conditions that occur within their valves (Holopainen & Penttinen, 1993). The unusual elliptical shape of bitterling eggs has a greater surface area to volume ratio than spherical eggs, which may enhance oxygen diffusion in low oxygen conditions. Furthermore, after embryonic hatching, the anterior portion of the yolk sac widens to form a wedge shape with two lateral elongations (Aldridge, 1999a), which may further increase the surface area to maximize oxygen diffusion. This wedge shape in the embryo, as well as the elliptical shape of eggs, may also make the mussel less able to dislodge bitterling from their gills.

Bitterling hatch at an early stage from the chorion (Aldridge, 1999a). Aldridge (1999a) suggested that as they do not require the protection of the chorion, which is aimed at embryos developing in open water, its early loss would enhance oxygen delivery. Finally, bitterling have an adaptation that is advantageous during periods of valve closure when potentially toxic levels of metabolic end-products would accumulate in an embryo. Instead of breaking down glycogen into lactate, which cannot be excreted into the surrounding water, bitterlings break glycogen down to ethanol and carbon dioxide, which can be excreted directly into water (Waarde *et al.*, 1993).

ADAPTIVE HOST PREFERENCES BY BITTERLING

HOST PREFERENCES IN RELATION TO MUSSEL SPECIES

Experiments have shown that bitterling choose to spawn in mussel species in which the survival of their offspring is highest (Mills & Reynolds, 2002a). This study, of a population that had been introduced into England *c.* 100 years ago, showed that all four species of mussels are capable of ejecting bitterling eggs and embryos prematurely. When given a choice, both male and female bitterling chose mussels in the following order: *U. pictorum*, *U. tumidus*, *A. anatina* and *A. cygnea*. When the fish were not given a choice, this order was also the same as the rank order of numbers of eggs that the fish deposited into each mussel species per spawning. Finally, the fish also spawned more quickly into *U. pictorum*, with an intermediate spawning time into *U. tumidus* and *A. anatina*, and they took the longest time to spawn in *A. cygnea*. These three indices of spawning preferences were in the same rank order as the probability of mussels ‘accepting’ eggs (*i.e.* not ejecting them prematurely), when the fish were not given a choice among mussels (Mills & Reynolds, 2002a).

The findings from these laboratory experiments on mussel species choice matched the order of productivity (the number of live bitterling released from a mussel) among mussel species from the same population of fish in the English study site (Reynolds *et al.*, 1997). They also match the rank order of productivity from a population in the Czech Republic, which is in the centre of *R. sericeus*’ natural range (Smith *et al.*, 2000a). Furthermore, in an experiment with the Czech population that varied the numbers of embryos deposited into mussels, it was found that density-dependent mortality was highest in the least preferred and least productive mussel, *A. cygnea*, and lowest in *A. anatina* and *U. pictorum* (Smith *et al.*, 2000a). Tests in the field agreed with the laboratory tests in England in showing that the fish avoid *A. cygnea*, but there were no significant differences in preferences for the other three species.

There is evidence that the cues for choice of mussel species could be oxygen concentrations and ventilation rates of water coming from the exhalant siphon. Circumstantial evidence for oxygen content being important comes from a study of differences among mussel species in their consumption of oxygen (Smith *et al.*, 2001). There is good reason to expect bitterling to be sensitive to oxygenation of their embryos, as this service is provided entirely by the flow of water that they receive inside the mussel’s gills. The fish could be sensitive to oxygen rates themselves, as well as the mussel’s ventilation rate, *i.e.* the rate at which water is pumped through its gills. The use of ventilation rates for selecting individual mussels was shown experimentally by Mills & Reynolds (2002b). When given a choice, female bitterling preferred mussels that had high flow speeds and pumped a larger volume of water per unit time. These results were also found in experiments with artificial mussels, in which ventilation rate (but not oxygenation of the water) was manipulated (Mills & Reynolds, 2002b). Taken together, these studies therefore suggest that bitterling are sensitive to the flow of water and its oxygen content as it leaves the mussel, and this may explain why females inspect mussels by facing downwards over the exhalant siphon.

Host preferences based on mussel ventilation rates may have benefits in addition to oxygenation of embryos. High ventilation rates are associated with a lack of super-parasitism, as bitterling embryos reduce mussel ventilation rates (S.C. Mills & J.D. Reynolds, unpubl. data). Preferences for such mussels may therefore avoid the negative effects of large numbers of embryos on per capita offspring survival (Smith *et al.*, 2000a). Preferences for high ventilation rates would also be advantageous in adverse environmental conditions, as high concentrations of phosphates, as well as low concentrations of algae and oxygen, reduce the amount of time that mussels spend ventilating and increase bitterling embryo mortality (Reynolds & Guillaume, 1998; pers. obs.).

HOST PREFERENCES IN RELATION TO PRIOR PRESENCE OF BITTERLING EMBRYOS

Bitterling show preferences for mussels based on the number of embryos already in a mussel's gills (Smith *et al.*, 2000a, 2002; Candolin & Reynolds, 2001). In choice experiments between mussels with and without bitterling embryos, most females avoided spawning into mussels with embryos, and of those that did spawn, females spawned more quickly into mussels without embryos (Candolin & Reynolds, 2001). Similarly, in choice experiments between 'high' and 'low' quality mussels (based on low or high numbers of bitterling embryos in their gills respectively), female bitterling spawned at a higher rate into 'high' quality mussels (Smith *et al.*, 2000a, 2002). Whereas Candolin & Reynolds (2001) found no change in either male bitterling courtship behaviour or colouration in relation to the presence of embryos in mussels, Smith *et al.* (2002) found that male bitterling led females at a higher rate towards mussels containing fewer embryos. Preferences for mussels containing fewer bitterling embryos are adaptive as both experimental and field methods have shown that embryo mortality is density dependent (Smith *et al.*, 2000a).

HOST PREFERENCES IN RELATION TO MUSSEL SEX AND REPRODUCTION

Females of all four mussel species that bitterling use as hosts incubate their own larvae (glochidia) within their gills. Whereas bitterling embryos may be deposited in either the inner or outer pair of gills, glochidia are always incubated in the outer gills. Spawning bitterling may be expected to be sensitive to the presence of glochidia in the gills for several reasons. First, glochidia consume considerable amounts of oxygen, and they cause reductions in mussel ventilation rates (Tankersley & Dimock, 1993; Tankersley, 1996). Glochidia may therefore interfere directly with the two main cues that fish probably use to select mussels, and from the bitterling's perspective, avoidance of such mussels may be adaptive. On the other hand, brooding female mussels could be less likely to expel bitterling prematurely if they risk expelling their own glochidia at the same time. The second reason why bitterling host choices might be affected by presence of glochidia is that female fish may be physically prevented from spawning into gills that carry glochidia because the mussels' water tubes, which are modified in the outer gills for glochidia incubation, become capped at their dorsal end (Tankersley & Dimock, 1993; Fig. 1).

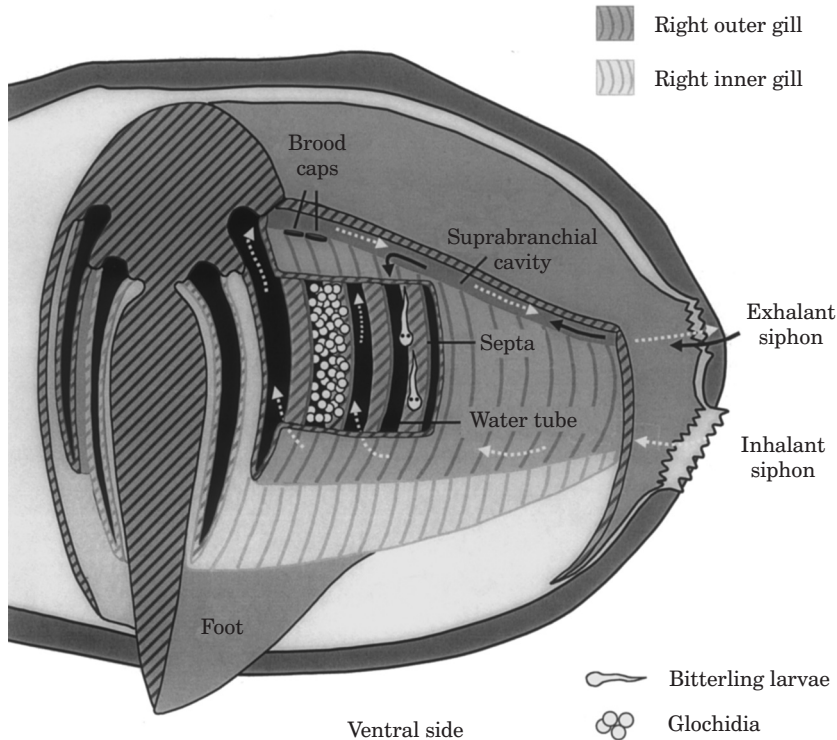




FIG. 1. Diagrammatic cross section through the gills and foot of a mussel. Diagonal shading represents the cross sectional areas. This diagram shows how each gill is compartmentalized dorso-ventrally into water tubes. , the flow of water currents within a mussel; , the direction that bitterling eggs are laid into a mussel.

A new study was initiated to investigate whether there were differences in host preferences and in bitterling productivity between the inner and outer gills of mussels depending on whether the mussel was a male or female and, in the case of females, according to whether or not it was incubating glochidia. Specifically, three questions were asked: (1) Do bitterling oviposit differentially into the inner and outer gills of mussels according to the mussels' reproductive state? (2) Do any such differences match the distribution of offspring in mussels in the wild? (3) Could differences among mussels in ventilation rates provide information about their sex and reproductive state?

Experiment 1. Bitterling egg deposition in relation to mussel sex and reproductive state

Rhodeus sericeus and 10 individuals of each of the four mussel species, *U. pictorum*, *U. tumidus*, *A. anatina* and *A. cygnea* were collected in March 1999 from a study site in Reach Lode, a tributary of the River Cam, Cambridgeshire, England (Reynolds *et al.*, 1997). One mussel in a sand-filled round glass container (10 × 6 cm) was placed in the centre of the 150 l aquarium. One male bitterling in full breeding colouration was placed with one female that had a long transparent ovipositor. Observations were made until the fish pair had spawned twice in the

mussel. The mussel was then removed and a mussel of a different species was added until the pair had spawned twice in all four species of mussel. The sequence in which the mussels were presented was alternated among replicates. Ten replicates of this experiment, *i.e.* 10 pairs of fish, were obtained. The mean \pm s.e. mussel lengths were as follows: *U. pictorum* = 81.3 ± 2.8 , *U. tumidus* = 72.6 ± 3.5 , *A. anatina* = 93.0 ± 2.2 and *A. cygnea* = 86.7 ± 4.5 mm.

After the fish spawned, the mussels were frozen and later dissected to establish their sex, their reproductive stage, and to count the number of bitterling eggs in the inner and outer gills. As it was only possible to ascertain the sex and reproductive state of the mussels after dissection, a randomised block design for mussel sex and reproductive state could not be carried out. All four species of mussel were used, however, to increase the chances that each fish pair would be presented with mussels that differed in sex and stage of reproduction.

Experiment 2. Bitterling embryo distribution among gills and mussels in the wild

Ten *U. pictorum*, nine *A. anatina* and six *U. tumidus* were collected on 25 May 2001. The mussels were dissected and the number of 'older' bitterling larvae present in the inner and outer gills was counted. These older larvae had eyes that were pigmented, the yolk sac was nearly fully absorbed and they were 8 mm in length. These bitterling larvae would have been *c.* 21 days old and would have left the mussels within *c.* 7 days (Aldridge, 1999a). The mussels' sex and reproductive stage were also recorded.

Experiment 3. Ventilation rates of Unio pictorum in relation to sex and reproduction

Twenty individuals of *U. pictorum* were collected during March and April 2000 and maintained in outdoor pools at the University of East Anglia. On 16 May 2000, the mussels were placed in sand-filled (10 cm deep) plastic troughs (75 \times 22 cm and 17 cm high) covered with a large piece of plastic netting with 1.5 cm² holes at 0.5 cm distances, so that they were inaccessible to the fish, and then returned to the study site to acclimatize. After 1 week the ventilation rates of each mussel were measured at the site according to the methods described in Mills & Reynolds (2002b). The mussels were dissected to establish their sex and reproductive condition. The ventilation rates were compared among males, pre-brooding and brooding females.

Results of these experiments

In experiment 1 no difference in the total number of eggs laid was found after two spawnings among male and female mussels at different stages of reproduction, among mussel species, or among fish pairs (univariate model, reproductive state, d.f. = 3 and 38, $P = 0.33$; mussel species, d.f. = 3 and 38, $P = 0.25$; fish pair, d.f. = 12 and 38, $P = 0.56$). There was a difference, however, in the distribution of these eggs between the inner and the outer gills among males and females at different stages of reproduction [univariate model, inner: d.f. = 3 and 38, $P < 0.01$; outer: d.f. = 3 and 38, $P < 0.05$; Fig. 2(a)]. When female mussels were brooding their own offspring (always in the outer gills), bitterling eggs were laid in the inner gills (paired *t*-test, $n = 9$, $P < 0.001$). The reverse occurred in mussels that had released their own larvae ($n = 10$, $P < 0.032$).

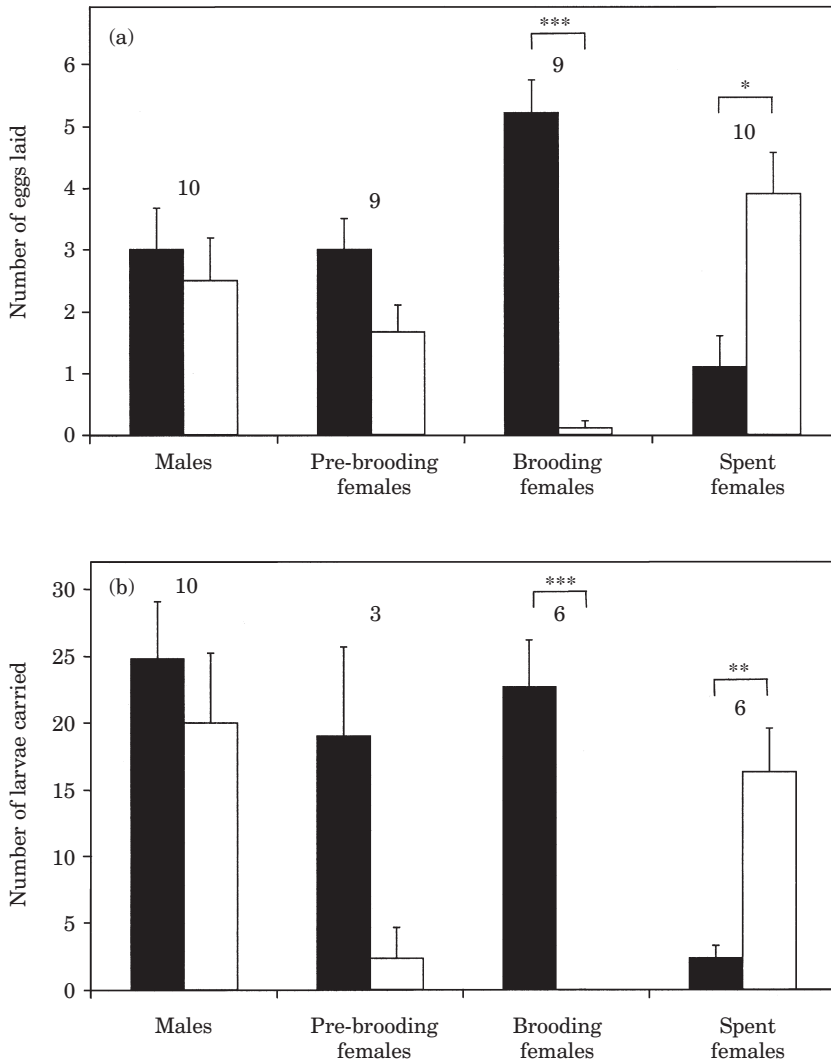


FIG. 2. The mean \pm S.E. number of bitterling (a) eggs laid in the laboratory and (b) bitterling larvae carried in wild mussels between the inner (■) and outer (□) gills. Numbers above bars are the sample sizes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In experiment 2 a good correspondence was found between spawning choices in the laboratory [Fig. 2(a)] and the number of 'older' bitterling larvae that had absorbed their yolk sac and were within *c.* 1 week of leaving the mussel in the wild [Fig. 2(b)]. As in the laboratory experiment, there was a difference in the distribution of bitterling larvae between the inner and outer gills among males and females at different stages of reproduction (univariate model, inner: d.f. = 3 and 25, $P = 0.055$; outer: d.f. = 3 and 25, $P < 0.001$; [Fig. 2(b)]. The bitterling larvae were found in the inner gills when females were brooding their own larvae (paired *t*-test: $n = 6$, $P = 0.001$), and in the outer gills of spent females ($n = 6$, $P = 0.005$).

More fish larvae were found in the gills of male mussels than in either brooding or spent female mussels (univariate model, d.f. = 2 and 25, $P = 0.019$; Tukey *post hoc*: $P = 0.015$). More bitterling larvae were also found in the gills of *U. pictorum* than in *A. anatina* (d.f. = 2 and 25, $P = 0.025$; Tukey *post hoc*: $P = 0.017$), however, there was no significant effect of the interaction between reproductive state and mussel species (d.f. = 1 and 25, $P = 0.27$).

Experiment 3 showed that the ventilation rates of female mussels that were brooding glochidia were significantly lower than those of non-brooding females and males (one-way ANOVA, d.f. = 2 and 19, $P = 0.005$; Tukey *post hoc*: $P = 0.01$ and $P = 0.008$ respectively; Fig. 3). There was no significant difference between the ventilation rates of male mussels and non-brooding female mussels (Tukey *post hoc*: $P = 0.83$).

Adaptive host preferences in relation to mussel reproductive state?

This study shows significant differences in the way female bitterling distribute their eggs between a mussel's inner and outer pairs of gills, depending on the mussel's sex and reproductive state [Fig. 2(a)]. These choices match the distribution of mature bitterling larvae in mussels from the wild [Fig. 2(b)]. The ventilation rates of mussels depended on their sex and reproductive state (Fig. 3) and may therefore provide a cue for the fish during oviposition.

There was a striking switch in the position of eggs from the inner gills when mussels were brooding glochidia (in their outer gills) to the outer gills after mussels had released their glochidia. The switch to outer gills was not necessarily due to an adaptive decision by female bitterling. This is because the water tubes of the outer gills are considerably swollen when brooding glochidia and the suprabranchial cavities remain enlarged for a few weeks following glochidia release (Tankersley & Dimock, 1992; Tankersley, 1996). Therefore, the possibility that a bitterling's ovipositor may enter the cavity of the outer gill of spent

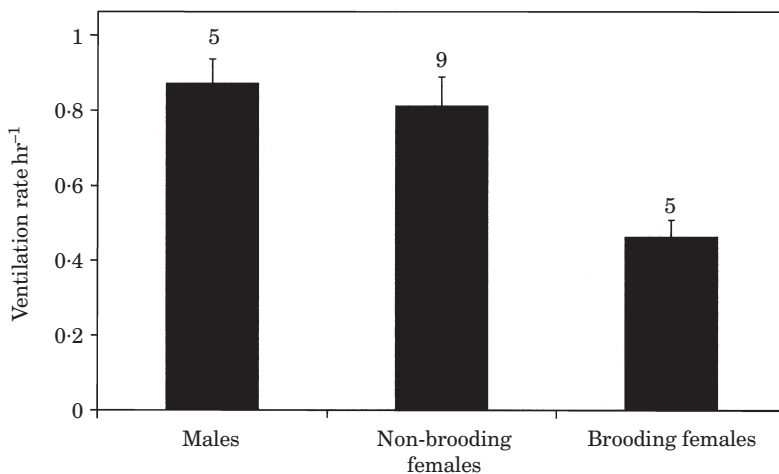


FIG. 3. The mean + s.e. ventilation rates of male *Unio pictorum* mussels and non-brooding and brooding female *U. pictorum* mussels. Numbers above bars are the sample sizes.

females more often simply as a result of the cavity's enlarged size cannot be ruled out.

The differential spawning by female bitterling into the inner gills of brooding mussels cannot be explained simply by the presence of caps that block the outer gills of these mussels. This is because the four gills of a freshwater mussel *Pyganodon cataracta* (formerly *Anodonta cataracta*) open into suprabranchial cavities that are isolated from one another until they meet at the base of the exhalant siphon (Tankersley & Dimock, 1993) (Fig. 1). If ovipositions were non-selective, the ovipositor would enter the four suprabranchial cavities at random and half of the spawnings would lead to eggs being blocked from entering the gills by the caps. In fact, as suggested above, the ovipositor might enter the enlarged suprabranchial cavities of the brooding outer gills more often if spawning was by chance, resulting in an even greater number of eggs blocked by the caps. Dissections did not find this (Fig. 2). Instead, they confirmed that all females laid eggs when they inserted their ovipositors, and all of the eggs ended up in gills. Therefore, the distribution of eggs in the inner gills of gravid females [Fig. 2(a)] suggests that female bitterling are able somehow to position their ovipositors down a suprabranchial cavity of choice. During the 1–2 s spawning act female bitterling do not give any sign of probing once inside the mussel, and given the non-muscular nature of the ovipositor this seems highly unlikely anyway.

It is worth considering whether female bitterling could choose a gill for its eggs based on the ventilation rate of the suprabranchial cavity. Brooding female mussels in the present study had reduced overall ventilation rates, in agreement with studies of the freshwater mussel *P. cataracta* (Tankersley & Dimock, 1993; Tankersley, 1996). This is because the capped water tubes of brooding outer gills prevent the flow of water through the gills, resulting in a lower total ventilation rate in gravid mussels (Tankersley & Dimock, 1992). The ventilation rate of outer gills in spent mussels, however, returns to its pre-brooding level (Tankersley & Dimock, 1993), suggesting that bitterling would not be able to use this as a means of distinguishing between the inner and outer gills of spent mussels.

Although secondary water tubes are formed in brooding outer gills, they only partially compensate for the capped water tubes and Tankersley & Dimock (1992) predicted that the total flow in marsupial (female outer) gills during brooding would be *c.* 16% of that in non-gravid marsupial gills and 4% of that in non-marsupial gills respectively. As the pair of outer gills represent 50% of the total pumping rate of mussels, these findings suggest that the total pumping rate of gravid females would be 58 and 52% of the pumping rate in non-brooding females and males respectively. The comparable measurements in species used in the present study are strikingly similar, at 57 and 53% respectively.

In conclusion, bitterling change the distribution of their eggs between inner and outer gills of female mussels at different stages of the mussels' reproductive cycle. These findings on oviposition behaviour within mussels complement those from previous studies showing adaptive preferences for different mussel species, and for mussels that contain low numbers of embryos from previous spawnings. The fish avoid the capped outer gills of mussels that are brooding their own young, but switch to the outer gills after the mussels have released their glochidia.

SELECTION ACTING ON MUSSELS

COSTS TO MUSSELS FROM BITTERLING

The presence of large numbers of bitterling eggs in the gills of mussels (well over 100 eggs have been recorded) visibly distorts the gills and significantly decreases the mussel's ventilation rate (S.C. Mills & J.D. Reynolds, unpubl. data). This reduction in water transport may have severe consequences for filter feeding and oxygen uptake (Tankersley, 1996), which could translate into costs of mussel growth, reproduction and survival.

Currently, there is no direct evidence of reductions in ventilation rates translating into fitness costs to mussels, unlike the high costs that have been documented for foster parents in other species, such as cuckoos and cowbirds (Rothstein, 1975a; Davies & Brooke, 1988; Davies, 2000). The experiments in the previous section showed that bitterling embryos do not compete directly with brooding glochidia for space, as the embryos and glochidia are carried in different gills. Smith *et al.* (2001), however, have shown that bitterling embryos reduce oxygen in the mussels. For example, the presence of 50 embryos, which is well below the maximum, can lead to the removal of *c.* 20% of the oxygen when the embryos are 25 days old. Embryos may therefore reduce oxygen available for glochidia. This effect is apt to be magnified by the fact that ventilation rates (which determine the rate at which oxygen arrives) are reduced in mussels both brooding glochidia (Fig. 3) and incubating bitterling (S.C. Mills & J.D. Reynolds, unpubl. data).

BENEFITS TO MUSSELS FROM BITTERLING

Glochidia

The glochidia of freshwater unionids, including all four species of mussels that are used by *R. sericeus*, are obligate parasites that attach their glochidia to the gills and fins of fishes (Baer, 1952; Wood, 1974; Kat, 1984). Freshwater unionids release more of their offspring in the presence of fishes, responding to tactile, chemical or visual stimuli (Jokela & Palokangas, 1993). Bitterling therefore represent a potentially ideal host to unionid glochidia and some popular books claim that bitterling are hosts to the glochidia of unionid mussels, implying a mutualistic relationship (Wheeler, 1983; Phillips & Rix, 1985). Aldridge (1997), however, showed that out of five commonly occurring fish species experimentally infected with glochidia, bitterling had the lowest number of glochidia attached. No *A. cygnea* glochidia successfully attached to bitterling and all *A. anatina* glochidia were lost within 5 days, whereas individual perch *Perca fluviatilis* L. carried up to 650 attached glochidia. During 11 years of studying bitterling, no glochidia have been found attached to fish caught in the wild, nor when glochidia have been released by mussels during fish spawning experiments in the laboratory (unpubl. obs.; see also Kadlec *et al.*, 2003).

Similar results have been found in species closely related to *R. sericeus*. In Hong Kong, the glochidia of *Anodonta woodiana* are very similar to that of *A. cygnea* from Europe, and only 10% of *Rhodeus sinensis* Günther were found with glochidia attached and of these, only one glochidium was found per host fish (Dudgeon & Morton, 1984). In Osaka, Japan, it was found that

A. woodiana mostly parasitized *Rhinogobius brunneus* (Temminck & Schlegel), while *Rhodeus ocellatus ocellatus* (Kner) was a poor host with less than one glochidium per fish (Fukuhara *et al.*, 1986). Therefore, contrary to the idea that the bitterlings and mussels parasitize each other, the evidence suggests that *Rhodeus* spp. are not suitable hosts for mussel glochidia.

BITTERLING SPERM

During courtship and oviposition male bitterling release sperm clouds over the inhalant siphon of mussels. It is common for sneaking to occur, whereby several non-territorial males release sperm immediately after a female spawns (Candolin & Reynolds, 2002*a,b*; Mills & Reynolds, 2003, Smith *et al.*, 2002). During the peak of the spawning season, >20 males have been observed releasing sperm over the mussel (unpubl. obs.). Can mussels ingest bitterling sperm, and thereby benefit from enhanced growth? The following are new results that test this hypothesis, based on an experiment that examined impacts of sex ratios of males on the bitterling's mating system (Mills & Reynolds, 2003).

Methods

In March 2000 40 individuals of *U. pictorum* were collected by hand from Reach Lode, Cambridgeshire. The external dimensions of the mussels' shells were measured to the nearest mm and mussels were weighed to the nearest g, then the mussels were paired according to similar shell dimension and mass. Twenty pools (140 × 90 cm and 30 cm high) covered in a 10 cm layer of washed sand containing de-chlorinated fresh water, were divided in half using netting and each pair of mussels was randomly assigned to each pool, one mussel per pool half. A rectangular retainer (80 × 40 cm and 10 cm high) was positioned centrally in each half of the pool to maintain the mussel within a fixed area.

Bitterling of mixed sex were caught from the same site as the mussels. One half of each pool contained a low male density treatment consisting of three males (mean ± s.e. standard body length, $L_S = 45.5 \pm 5.2$ mm) and three females (37.2 ± 7.3 mm). The other half contained a high male density treatment consisting of 18 males (40.1 ± 1.0 mm) and three females. Bitterling populations have been recorded to vary by over two orders of magnitude among lakes that vary in size by only four-fold in the Czech Republic (Smith *et al.*, 2000*b*).

The reproductive behaviour of both sexes of bitterling was recorded from 16 May to 30 June 2000. Behavioural observations of event frequency data were made over a 10 min period in each pool with a 5 min delay between pools. Frequency estimates of the following reproductive behaviours were made for large, medium and small males and females: sperm release (a white sperm cloud was visible), attempted spawning (female missed the mussel siphons with ovipositor), and successful spawning (ovipositor momentarily rigid in exhalant siphon).

On 1 September 2000, 5 months after the onset of the experiment, the shell parameters of the mussels were re-measured as was the total wet-mass of the mussels. The internal body parts were dissected and dried at 66°C for 48 h before weighing the following to the nearest 0.001 g: outer and inner gills, gonads, adductor muscles, foot and the remaining internal matter.

Results

As expected there were more sperm releases by male bitterling over the inhalant siphon of mussels in the 20 half-pools that had the high male density treatment than in the 20 low male density replicates (mean \pm s.e. number of sperm releases: low male density = 1.5 ± 0.2 , high male density = 2.8 ± 0.3 ; paired *t*-test on the mean number of sperm releases, d.f. = 20, $P < 0.001$). There was no difference in the number of successful spawnings by female bitterling between the two treatments (Mills & Reynolds, 2003), nor was there a difference between the number of offspring released from mussels between the two treatments (paired *t*-test on the mean number of offspring released, $n = 10$, $P < 0.72$).

All the shell variables increased significantly during the 5 month experiment, irrespective of the male density treatment (Table I). Male density and the corresponding sperm release numbers had no significant effect on the differences in mussel shell growth over the experiment (Wilcoxon matched pairs signed-ranks test on the differences of the three shell variables over the experiment, length: $n = 19$, $P < 0.20$; height: $n = 19$, $P < 0.08$; breadth: $n = 19$, $P < 0.45$). Furthermore, sperm release numbers had no significant effect on the dry masses of any of the internal body parts including the gonads (Table II).

TABLE I. Paired *t*-tests on mussel shell variables between the beginning of the experiment and the end of the experiment within low and high male bitterling density treatments

Variable	Before experiment mean \pm s.e.	After experiment mean \pm s.e.	<i>t</i> (d.f. = 20)	<i>P</i>
Low male density:				
Length (mm)	70.4 \pm 1.5	71.1 \pm 1.5	5.00	<0.001
Height (mm)	28.1 \pm 0.5	28.4 \pm 0.6	3.58	<0.002
Width (mm)	16.8 \pm 0.4	17.0 \pm 0.4	3.56	<0.002
High male density:				
Length (mm)	70.5 \pm 1.4	71.3 \pm 1.5	7.39	<0.001
Height (mm)	27.7 \pm 0.6	28.4 \pm 0.7	5.90	<0.001
Width (mm)	17.0 \pm 0.4	17.2 \pm 0.4	3.01	<0.007

TABLE II. Paired *t*-tests on the dry masses of the internal body parts of mussels between low and high male bitterling density treatments

Dry masses	Low male density mean \pm s.e.	High male density mean \pm s.e.	<i>t</i> (d.f. = 19)	<i>P</i>
Outer gills	0.082 \pm 0.004	0.077 \pm 0.007	0.69	0.49
Inner gills	0.088 \pm 0.005	0.097 \pm 0.008	0.91	0.37
Foot	0.132 \pm 0.011	0.132 \pm 0.014	0.03	0.98
Muscles	0.073 \pm 0.006	0.071 \pm 0.007	0.34	0.74
Gonads	0.371 \pm 0.045	0.359 \pm 0.049	0.17	0.87
Rest of body	0.448 \pm 0.045	0.459 \pm 0.052	0.18	0.86

Do mussels benefit from bitterling?

The experiment does not support the hypothesis that mussels benefit from bitterling sperm through enhanced growth. While shell dimensions and the dry masses of various body parts increased over the 5 month course of the experiment, growth was not affected by the difference in the amount of sperm released by males in the two treatments. While sperm is clearly drawn into the gills (thereby fertilizing bitterling eggs), it is possible that mussels are unable to ingest sperm, or that the benefits of doing so are minimal.

THE FREQUENCY OF PARASITISM

The evolution of host defences should depend on both the strength of selection and on the frequency of parasitism (Davies *et al.*, 1989). In avian brood parasitism, theoretical and experimental evidence have shown that it may pay hosts to accept parasitic eggs when the probability of parasitism is low, but they should reject parasitic eggs when the probability of parasitism is high (Takasu *et al.*, 1993; Lotem *et al.*, 1995; Davies *et al.*, 1996; Lyon, 2003).

The frequency of parasitism by bitterling can be quite high. For example, at the study site in England, *U. pictorum* released an average of nine fish per mussel, followed by five fish per *A. anatina*, and one fish per mussel in *U. tumidus* and *A. cygnea* (Reynolds *et al.*, 1997). In another study of *A. anatina* from the same site, all mussels released at least one bitterling ($n = 35$ mussels) (Reynolds & Guillaume, 1998). At the Czech Republic study site, when mussels were transported from one small lake to another and exposed to bitterling for 6 days, *A. anatina* contained an average of 95 fish per mussel, followed by *U. pictorum* (83), *U. tumidus* (76) and *A. cygnea* (32) (Smith *et al.*, 2000a).

It would obviously be risky to generalize from these two study sites, but if they are representative of the typical frequency of parasitism elsewhere in the bitterling's range, they imply that the most popular species of mussels may face a high incidence of parasitism from bitterling. Therefore, where mussels coexist with bitterling, it cannot be inferred that low rates of rejection by species such as *U. pictorum* and *A. anatina* can be explained solely on the basis of low encounter rates with the fish.

HAVE BITTERLING AND MUSSELS CO-EVOLVED?

While there is evidence that bitterling preferences for mussel species match differences in survival of their young (Mills & Reynolds, 2002a), there is no experimental evidence that mussel behaviour has evolved to avoid parasitism by bitterling. One way in which reed warblers *Acrocephalus scirpaceus* avoid parasitism by cuckoos is to nest in areas with the lowest probability of being parasitized (Øien *et al.*, 1996). *Anodonta cygnea* generally inhabits deeper water than the other species, an area where bitterling are less commonly found. It is doubtful if this behaviour has evolved in response to presence of bitterling; comparisons of mussel habitat use over a wide geographical area that varies in presence or absence of bitterling would provide the best evidence.

Another way to avoid parasitism is by fish egg ejection, however, there is no experimental evidence that mussel ejection behaviour has evolved in direct

response to parasitism by bitterling. For example, ejection of eggs could be a manifestation of a general response by mussels to dislodge any foreign object from their gills. Similarly, it has been shown that mussels tend to ventilate less and close their valves for extended periods in certain unfavourable conditions such as the presence of high concentrations of phosphates, and that this leads to premature mortality of fish embryos (Reynolds & Guillaume, 1998). The mortality of such embryos, however, could simply be a side effect, rather than a specific tactic that has evolved to rid the gills of embryos during unfavourable conditions (Reynolds & Guillaume, 1998). A host defence should only be called a host defence if it reduces the impact of parasitism and if there is evidence to suggest that it has evolved in response to, or is currently maintained by, selection pressures arising from parasitism (Rothstein, 1990).

Possible evolutionary origins of host egg ejection and gill evacuation may include the expulsion of pseudo-faeces (Jørgensen, 1990) and the release of mature glochidia (Tankersley & Dimock, 1993). Pseudo-faeces are masses of undigested phytoplankton that are expelled periodically by mussels. Their ejection is attributable to rapid valve adduction causing sudden water flow changes (Tankersley & Dimock, 1992). Unionid mussels are also affected by other parasites, such as water mites, *Unionicola* spp., which inhabit their gills (Dimock, 1985). Therefore, while the use of this ejection behaviour may be an adaptation to the presence of bitterling eggs, it could also be a generalized defence behaviour and not attributable to any particular species of parasite. This situation would be a case of diffuse co-evolution rather than the pairwise (reciprocal) co-evolution that is shown in avian brood parasitism (Rothstein, 1990). Studies of co-evolution have yielded two main theories to explain differences in host specialization and responses of hosts to parasites: the evolutionary lag hypothesis (Rothstein, 1975*b*; Brooke & Davies, 1988; Davies & Brooke, 1989*a*; Soler & Møller, 1990) and the evolutionary equilibrium hypothesis (Zahavi, 1979; Rohwer & Spaw, 1988; Marchetti, 1992).

EVOLUTIONARY LAG HYPOTHESIS

This hypothesis suggests that hosts and parasites are not in evolutionary equilibrium with one another because of insufficient genetic variation or time for evolution to have fine-tuned preferences by the parasites or defences by the hosts (Rothstein, 1975*a,b*; Dawkins & Krebs, 1979; Davies & Brooke, 1989*a,b*; Moksnes *et al.*, 1990). Tests of this theory typically compare host defences or host preferences between host-parasite systems of different ages (Soler & Møller, 1990; Briskie *et al.*, 1992).

The bitterling–mussel interaction in the Cambridgeshire population is relatively new (*c.* 100 years), based on the probable time since bitterling are thought to have been introduced to this site from continental Europe (Mills & Reynolds, 2002*a*). In contrast, the bitterling and mussel species in the Czech Republic study site have a much longer history of sympatry (Ellis, 1978; Lelek, 1980). The evolutionary lag hypothesis predicts that in the U.K., defences by mussels could be weak, whereas they could be stronger in the Czech Republic. In both sites, however, bitterling avoid *A. cygnea*, and survival is lowest in that species (Smith

et al., 2000a; Mills & Reynolds, 2002a). It seems unlikely that egg ejection behaviour by *A. cygnea* evolved in such a short time in the U.K., given their generation time of at least 5 years. The definitive test of this theory would be to compare host defences in mussels from a range of sites that differ in history of exposure to bitterling.

The evolutionary lag hypothesis might act at a much smaller scale, due to differences in habitats of the four species of mussels. This could cause differences in the counter-defences that bitterling have evolved among the four mussel species. The fish are commonly found in shallow, well-vegetated areas close to the shore. Three of the host species (*U. pictorum*, *U. tumidus* and *A. anatina*) are also typically found in this habitat, whereas as noted above, *A. cygnea* usually occurs in deeper water, farther from shore. Therefore, bitterling may have had less contact with *A. cygnea*, which could have led to weaker selection for adaptation by bitterling to this mussel species (Reynolds *et al.*, 1997). For example, the larger size of *A. cygnea* compared to the other host species of comparable age (Aldridge, 1999b) may require longer ovipositors for bitterling to reach the gills. Indeed, *A. cygnea* in the Czech Republic sites are often >20 cm in length, which probably makes it physically impossible for bitterling to deposit eggs securely into their gills.

A variant of the evolutionary lag hypothesis is the idea that metapopulation dynamics might explain differences in host rejection and parasitism rates (Røskaft *et al.*, 2002). Specifically, the immigration of recruits from unparasitized populations (which lack host defence) to vacancies within parasitized populations could prevent the evolution of host defences in locations where parasites are common. Using cuckoo-host co-evolution as an example, Røskaft *et al.* (2002) suggested that the breeding habitat of host species determines the risk of parasitism, through proximity to trees which provide vantage points to cuckoos for finding host nests. All populations of a host species that breed close to trees would rapidly evolve rejection behaviour, whereas host species breeding far from trees would be acceptors. Gene flow from populations with low exposure to cuckoos to one with high exposure, however, would delay the evolution of rejection behaviour. For this idea to work in the bitterling-mussel system, spatial variation would be needed among mussel populations in their rate of contact with bitterling, as well as gene flow among those populations. It has been shown experimentally that there are indeed differences in susceptibility of mussels to bitterling, according to their distance from shore (Reynolds *et al.*, 1997). It would be risky, however, to generalize from such fine-scale spatial variation into conclusions about genetic divergence and gene flow among mussels. The process could not work in small bodies of water such as those that were studied in England and the Czech Republic, but there could be other locations where it might.

Although the evolutionary lag hypothesis to explain differences in host specialization and defence in the bitterling-mussel system cannot be excluded, the evidence for it is weak at best. In addition to the difficulty for this hypothesis to explain the patterns of defence by *A. cygnea*, it does not explain greater rejection rates by *A. anatina* than by *U. pictorum*, which have geographical distributions and habitats that overlap to a similar extent with bitterling.

EVOLUTIONARY EQUILIBRIUM HYPOTHESIS

Under the equilibrium view, host defences incur costs which exceed the benefits of a defence (Zahavi, 1979; Rohwer & Spaw, 1988; Lotem *et al.*, 1992; Røskaft & Moksnes, 1998). Therefore, contrary to the evolutionary lag hypothesis, lack of host defence may not be due to lack of time or encounter frequency for the evolution of defences, but it may be due to an evolutionary equilibrium between hosts and parasites, with the hosts' defences constrained by costs.

One potential cost to mussels of ejecting bitterling eggs could be a risk of ejecting their own glochidia prematurely. This would be analogous to the risk that some species of birds face from accidentally breaking their own eggs when rejecting eggs of cowbirds or cuckoos (Davies, 2000). The period of glochidial incubation for *Unio* species coincides more with the bitterling spawning period than for *Anodonta* species. Therefore, *Unio* species may have more to risk from evolving a host defence (Mills & Reynolds, 2002a). This risk, however, could only apply to female mussels, and males of the *Unio* species would not be constrained from evolving a host defence. It has been shown in the present paper that there were no differences in the number of eggs spawned into male and female mussels, and male mussels incubated significantly more mature bitterling larvae (within 7 days of leaving the mussel) than females. Therefore, glochidial incubation does not appear to be the factor determining whether egg ejection evolves in *Unio* species.

A better reason why bitterling egg ejection might be costly for *Unio* species is that bitterling eggs are lodged more deeply in the gills of these species (Mills & Reynolds, 2002a). The bitterling–mussel interaction may represent a co-evolutionary asymmetry, similar to that shown by Moksnes *et al.* (1991) between different Norwegian cuckoo hosts where the size of the hosts' bill determines its ability to eject artificial common cuckoo *Cuculus canorus* eggs. In the bitterling–mussel interaction there may be constraints on the evolution of host defences by *U. pictorum* and *U. tumidus* because of greater difficulties ejecting bitterling eggs than for the larger mussel species *A. anatina* and *A. cygnea*.

Therefore, based on what is currently known about the costs and benefits of ejection, apart from potential physical constraints on *Unio* species, it is difficult to use the evolutionary equilibrium hypothesis to explain differences between mussel species in egg ejection.

CONCLUSIONS

There is evidence that spawning preferences of the European bitterling may have evolved in response to differences in mussel egg ejection rates. Indeed, bitterling make sophisticated choices according to the mussels' lack of embryos and reproductive state. There is also evidence that ventilation rates are reduced by the presence of fish embryos, and these costs are not known to be counter-balanced by any benefits. There is no evidence yet, however for a reciprocal adaptation by mussels to parasitism by bitterling. Neither the evolutionary lag, nor the evolutionary equilibrium hypothesis provides entirely satisfactory explanations for the patterns of host preference and host defence that have been documented.

The behaviour of egg ejection by mussels may not have evolved primarily in response to bitterling, as it is also used in pseudo-faeces expulsion and glochidia release. The success of ejection in expelling bitterling may therefore be related to aspects of host physiology. Therefore, on the basis of current evidence, the bitterling–mussel interaction may not be driven strongly by co-evolutionary dynamics. Instead, the system may be more one-sided, with bitterling having evolved a suite of oviposition preferences that include choice of mussel species and of individuals within species. In contrast, inherent differences in mussel physiology may be the prime determinants of their ejection response.

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References

- Aldridge, D. C. (1997). Reproductive ecology of bitterling (*Rhodeus sericeus* Pallas) and unionid mussels. PhD thesis, University of Cambridge.
- Aldridge, D. C. (1999a). Development of European bitterling in the gills of freshwater mussels. *Journal of Fish Biology* **54**, 138–151. doi: 10.1006/jfbi.1998.0850.
- Aldridge, D. C. (1999b). The morphology, growth and reproduction of Unionidae (Bivalvia) in a fenland waterway. *Journal of Molluscan Studies* **65**, 47–60.
- Anderson, R. M. & May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology* **85**, 411–426.
- Baer, J. G. (1952). Mollusca. In *Ecology of Animal Parasites* (Baer, J. G., ed.), pp. 20–22. Urbana, IL: University of Illinois Press.
- Briskie, J. V., Sealy, S. G. & Hobson, K. A. (1992). Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution* **46**, 334–340.
- Brooke, M. de L. & Davies, N. B. (1988). Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* **335**, 630–632.
- Candolin, U. & Reynolds, J. D. (2001). Sexual signaling in the European bitterling: females learn the truth by direct inspection of the resource. *Behavioral Ecology* **12**, 407–411.
- Candolin, U. & Reynolds, J. D. (2002a). Why do males tolerate sneakers? Tests with the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology*, **51**, 146–152.
- Candolin, U. & Reynolds, J. D. (2002b). Adjustments of ejaculation rates in response to risk of sperm competition in a fish, the bitterling (*Rhodeus sericeus*). *Proceedings of the Royal Society of London B* **269**, 1459–1553.
- Cunha, C., Mesquita, N., Dowling, T. E., Gillies, A. & Coelho, M. M. (2002). Phylogenetic relationships of Eurasian and American cyprinids using cytochrome *b* sequences. *Journal of Fish Biology* **61**, 929–944. doi: 10.1006/jfbi.2002.2105.
- Davies, N. B. (2000). *Cuckoos, Cowbirds and Other Cheats*. London: Poyser.
- Davies, N. B. & Brooke, M. de L. (1988). Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour* **36**, 262–284.
- Davies, N. B. & Brooke, M. de L. (1989a). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *Journal of Animal Ecology* **58**, 207–224.
- Davies, N. B. & Brooke, M. de L. (1989b). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *Journal of Animal Ecology* **58**, 225–236.

- Davies, N. B., Bourke, A. F. G. & Brooke, M. de L. (1989). Cuckoos and parasitic ants-interspecific brood parasitism as an evolutionary arms race. *Trends in Ecology & Evolution* **4**, 274–278.
- Davies, N. B., Brooke, M. de L. & Kacelnik, A. (1996). Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London Series B* **263**, 925–931.
- Dawkins, R. & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London Series B* **205**, 489–511.
- Dimock, R. V., Jr. (1985). Population dynamics of *Unionicola formosa* (Acari: Unionicolidae), a water mite with a harem. *American Midland Naturalist* **114**, 168–179.
- Dudgeon, D. & Morton, B. (1984). Site selection and attachment duration of *Anodonta woodiana* (Bivalvia, Unionacea) glochidia on fish hosts. *Journal of Zoology* **204**, 355–362.
- Duyvene de Wit, J. J. (1966). Some observations on the European Bitterling (*Rhodeus amarus*). *Suid-Afrikaanse Joernaal van Wetenskap* **51**, 249–251.
- Ellis, A. E. (1978). *A Synopsis of the British Freshwater Bivalve Mollusca*. London: Academic Press.
- Freyhof, J. (1997). Zur Fortpflanzungsbiologie des Barbengründlings. *Die Aquarien und Terrarien Zeitschrift* **50**, 513–516.
- Fukuhara, S., Nagata, Y. & Yamada, T. (1986). Glochidium parasitic period, host-fish and parasitic site of *Anodonta woodiana* in small pond. *Japanese Journal of Malacologia* **45**, 43–52.
- Heschl, A. (1989). Integration of “innate” and “learned” components within the IRME for mussel recognition in the European bitterling *Rhodeus amarus* (Bloch). *Ethology* **81**, 193–208.
- Holopainen, I. J. & Penttinen, O. P. (1993). Normoxic and anoxic heat output of the freshwater bivalves *Pisidium* and *Sphaerium*. *Oecologia* **93**, 215–223.
- Jokela, J. & Palokangas, P. (1993). Reproductive tactics in *Anodonta* clams – parental host recognition. *Animal Behaviour* **46**, 618–620.
- Jørgensen, C. B. (1990). *Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology*. Fredensborg: Olsen and Olsen.
- Kadlec, D., Šimková, A., Jarkovský, J. & Gelnar, M. (2003). Parasite communities of freshwater fish under flood conditions. *Parasitology Research* **89**, 272–283.
- Kat, P. W. (1984). Parasitism and the Unionacea (Bivalvia). *Biological Reviews of the Cambridge Philosophical Society* **59**, 189–207.
- Kramer, K. J. M., Jenner, H. A. & de Zwart, D. (1989). The valve movement response of mussels: a tool in biological monitoring. *Hydrobiologia* **188/189**, 433–443.
- Lelek, A. (1980). *Threatened Freshwater Fishes of Europe*. Strasbourg: Council of Europe.
- Lotem, A., Nakamura, H. & Zahavi, A. (1992). Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behavioral Ecology* **3**, 128–132.
- Lotem, A., Nakamura, H. & Zahavi, A. (1995). Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour* **49**, 1185–1209.
- Lyon, B. E. (2003). Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* **422**, 495–499.
- Maitland, P. S. & Campbell, R. N. (1992). *Freshwater Fishes*. London: Harper Collins.
- Marchetti, K. (1992). Costs of host defense and the persistence of parasitic cuckoos. *Proceedings of the Royal Society of London Series B* **248**, 41–45.
- Mills, S. C. & Reynolds, J. D. (2002a). Host species preferences by bitterling, *Rhodeus sericeus*, spawning in freshwater mussels and consequences for offspring survival. *Animal Behaviour* **63**, 1029–1036. doi: 10.1006/anbe.2001.1988.
- Mills, S. C. & Reynolds, J. D. (2002b). Mussel ventilation rates as a proximate cue for host selection by bitterling, *Rhodeus sericeus*. *Oecologia* **131**, 473–478. doi: 10.1007/s00442-002-0895-7.
- Mills, S. C. & Reynolds, J. D. (2003). Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology and Sociobiology* **54**, 98–104. doi: 10.1007/s00265-003-0616-1.

- Moksnes, A., Røskaft, E., Braa, A. T., Korsnes, L., Lampe, H. M. & Pedersen, H. C. (1990). Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* **116**, 64–89.
- Moksnes, A., Røskaft, E. & Braa, A. T. (1991). Rejection behavior by common cuckoo hosts towards artificial brood parasite eggs. *Auk* **108**, 348–354.
- Øien, I. J., Honza, M., Moksnes, A. & Røskaft, E. (1996). The risk of parasitism in relation to the distance from reed warbler nests to cuckoo perches. *Journal of Animal Ecology* **65**, 147–153.
- Phillips, R. & Rix, M. (1985). *Freshwater Fish of Britain, Ireland and Europe*. London: Pan Books.
- Reynolds, J. D. & Guillaume, H. P. (1998). Effects of phosphate on the reproductive symbiosis between bitterling and freshwater mussels: implications for conservation. *Journal of Applied Ecology* **35**, 575–581.
- Reynolds, J. D., Debuse, V. J. & Aldridge, D. C. (1997). Host specialisation in an unusual symbiosis: European bitterlings spawning in freshwater mussels. *Oikos* **78**, 539–545.
- Rohwer, S. & Spaw, C. D. (1988). Evolutionary lag versus bill-size constraints: a comparative study of the acceptance of cowbird eggs by old hosts. *Evolutionary Ecology* **2**, 27–36.
- Røskaft, E. & Moksnes, A. (1998). Coevolution between brood parasites and their hosts: an optimality theory approach. In *Parasitic Birds and their Hosts, Studies in Coevolution* (Rothstein, S. I. & Robinson, S. K., eds), pp. 236–254. New York: Oxford University Press.
- Røskaft, E., Moksnes, A., Stokke, B. G., Moskát, C. & Honza, M. (2002). The spatial habitat structure of host populations explains the pattern of rejection behavior in hosts and parasitic adaptations in cuckoos. *Behavioral Ecology* **13**, 163–168.
- Rothstein, S. I. (1975a). Evolutionary rates and host defenses against avian brood parasitism. *American Naturalist* **109**, 161–176.
- Rothstein, S. I. (1975b). An experimental and teleonomic investigation of avian brood parasitism. *Condor* **77**, 250–271.
- Rothstein, S. I. (1990). A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics* **21**, 481–508.
- Rothstein, S. I. & Robinson, S. K. (Eds) (1998). *Parasitic Birds and their Hosts: Studies in Coevolution*. New York: Oxford University Press.
- Shine, R. (1978). Propagule size and parental care: the “safe harbour” hypothesis. *Journal of Theoretical Biology* **75**, 417–424.
- Smith, C. C. & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *American Naturalist* **108**, 499–506.
- Smith, C., Reynolds, J. D., Sutherland, W. J. & Jurajda, P. (2000a). Adaptive host choice and avoidance of superparasitism in the spawning decisions of bitterling (*Rhodeus sericeus*). *Behavioral Ecology and Sociobiology* **48**, 29–35.
- Smith, C., Reynolds, J. D. & Sutherland, W. J. (2000b). Population consequences of reproductive decisions. *Proceedings of the Royal Society of London Series B* **267**, 1327–1334.
- Smith, C., Rippon, K., Douglas, A. & Jurajda, P. (2001). A proximate cue for oviposition site choice in the bitterling (*Rhodeus sericeus*). *Freshwater Biology* **46**, 903–911.
- Smith, C., Douglas, A. & Jurajda, P. (2002). Sexual conflict, sexual selection and sperm competition in the spawning decisions of bitterling, *Rhodeus sericeus*. *Behavioral Ecology and Sociobiology* **51**, 433–439.
- Soler, M. & Møller, A. P. (1990). Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature* **343**, 748–750.
- Storey, K. B. & Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Quarterly Review of Biology* **65**, 145–174.
- Takasu, F. (1998). Modelling the arms race in avian brood parasitism. *Evolutionary Ecology* **12**, 969–987.

- Takasu, F., Kawasaki, K., Nakamura, H., Cohen, J. E. & Shigesada, N. (1993). Modeling the population dynamics of a cuckoo host association and the evolution of host defenses. *American Naturalist* **142**, 819–839.
- Tankersley, R. A. (1996). Multipurpose gills: Effect of larval brooding on the feeding physiology of freshwater unionid mussels. *Invertebrate Biology* **115**, 243–255.
- Tankersley, R. A. & Dimock, R. V. (1992). Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel *Anodonta cataracta*. *Biological Bulletin* **182**, 145–154.
- Tankersley, R. A. & Dimock, R. V. (1993). Endoscopic visualization of the functional morphology of the ctenidia of the Unionid mussel *Pyganodon cataracta*. *Canadian Journal of Zoology* **71**, 811–819.
- Thompson, J. N. (1994). *The Coevolutionary Process*. Chicago: The University of Chicago Press.
- Thompson, J. N. & Burdon, J. J. (1992). Gene-for-gene coevolution between plants and parasites. *Nature* **360**, 121–125.
- van Waarde, A., van den Thillart, G. & Verhagen, M. (1993). Ethanol formation and pH-regulation in fish. In *Surviving Hypoxia: Mechanisms of Control and Adaptation* (Hochachka, P. W., Lutz, P. L., Sick, T., Rosenthal, M. & van den Thillart, G., eds), pp. 157–170. Boca Raton, FL: CRC Press.
- Wheeler, A. (1983). *Freshwater Fishes of Britain and Europe*. London: Kingfisher Books.
- Wiepkema, P. R. (1961). An ethological analysis of the reproductive behaviour of the bitterling (*Rhodeus amarus* Bloch). *Archives Néerlandaises de Zoologie* **14**, 103–199.
- Wood, E. M. (1974). Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). *Journal of Zoology, London* **173**, 1–14.
- Zahavi, A. (1979). Parasitism and nest predation in parasitic cuckoos. *American Naturalist* **113**, 157–159.