



## ARTICLES

# Host species preferences by bitterling, *Rhodeus sericeus*, spawning in freshwater mussels and consequences for offspring survival

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Two hypotheses have been advanced to explain the evolution of host responses to parasites: the arms race–evolutionary lag and equilibrium hypotheses. We investigated predictions from these hypotheses based on interspecies host preferences and adaptations in an obligate spawning relationship between a freshwater fish, the European bitterling (Cyprinidae) and four species of freshwater mussels (Unionidae), which the fish use as hosts for their eggs. We found a significant trend in preference by the fish for mussels in the following order: *Unio pictorum*, *U. tumidus*, *Anodonta anatina* and *A. cygnea*. Male and female bitterling both showed this ranking and the clutch sizes deposited into each species also followed this trend. These host preferences proved to be adaptive in terms of egg ejection, which was lowest in the most preferred species (*U. pictorum*). Furthermore, these hierarchical host preferences were flexible, as females switched species when individuals of the preferred species ejected a greater number of eggs. The similarity in mussel defences between the U.K. population and a European population of ancient sympatry suggests that the absence of a defence in some mussel species may not be due to evolutionary lag. Mussel ejection behaviour may have reached an evolutionary equilibrium in each host species, or alternatively the fish may have evolved adaptive preferences that coincide with generalized mussel responses to foreign objects in their gills.

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Host–parasite interactions can take a number of forms including those causing infectious disease, such as *Plasmodium* species (Ewald 1994), insect host–parasitoids (Mayhew 2001), avian brood parasitism (Davies 2000) and egg dumping (Andersson & Ahlund 2001). An understanding of these host–parasite interactions has led to theoretical advances in our understanding of the evolution of adaptive traits and of coevolution (reviewed in Rothstein & Robinson 1998; Soler & Soler 2000). These theories seek to explain the evolution of host specialization and why hosts vary in their defences against parasites.

Host specialization may or may not be adaptive. The evolutionary lag hypothesis (Davies & Brooke 1989a; Rothstein 1990; Soler & Møller 1990) suggests that species

may not parasitize the best possible host because their history of encounter rates has been too short for them to evolve appropriate behaviour or physiology. For example, in the Rocky Mountains, U.S.A. pierid butterflies parasitize the crucifer food plant *Thlaspi arvense*, which is lethal to their larvae. This maladaptive behaviour has been attributed to the recent introduction of this plant into the region (Chew 1977). For the same reasons host responses need not be adaptive, if hosts have not had sufficient time or genetic variation to evolve counteradaptations. Host specialization may also evolve through a one-sided adaptation whereby animals evolve to use the best hosts, while hosts remain unchanged. Finally, adaptive host specialization may result from an equilibrium among various selection pressures: the evolutionary equilibrium hypothesis (Rohwer & Spaw 1988; Davies & Brooke 1989b; Marchetti 1992; Soler et al. 1995). For example, avian brood parasites may choose hosts that, because the costs outweigh the benefits, do not eject parasitic eggs.

Models of host–parasite coevolution have failed to produce a single parsimonious explanation for the

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different host responses to parasites (Takasu et al. 1993; Pagel et al. 1998; Takasu 1998). Differences between these hypotheses for coevolution are well illustrated by avian brood parasitism (Rothstein & Robinson 1998; Davies 2000). Under the evolutionary lag hypothesis a host that rejects parasites will have a greater lifetime reproductive success than an acceptor; however, under the evolutionary equilibrium hypothesis it is not necessarily adaptive for hosts to reject (Winfree 1999). To understand the evolution of host preference we need to ascertain the extent to which host choice reflects host adaptations and whether there has been reciprocal adaptive change in the interacting species.

We tested these ideas by comparing host preferences with offspring survival in the obligate spawning relationship between a species of freshwater fish, the bitterling, and four species of freshwater mussels. This interaction is ideal for this study as host preferences by fish of both sexes can be studied readily and spawnings can be manipulated to relate preferences to offspring survival. Bitterling are members of the subfamily Acheilognathinae (family Cyprinidae) and over much of their European range they share a common habitat with four species of freshwater Unionid mussels: *Unio pictorum*, *U. tumidus*, *Anodonta anatina* and *A. cygnea* (Reynolds et al. 1997; Smith et al. 2000). Males defend an area containing one or more mussels and court females. Females inspect the available mussels, choose between the mussels based partly on their ventilation rates (Mills & Reynolds 2002) and use their long ovipositors to force eggs through the exhalant siphon into the gills of the mussel host (Wiepkema 1961; Heschl 1989). Male bitterling fertilize eggs by releasing sperm, which is drawn into the mussel with the inhalant respiratory current (Duyvene de Wit 1966). Mussels incubate the eggs for 2–4 weeks, until the yolk sac is absorbed when the young bitterling swim out of the exhalant siphon of the living mussels (Reynolds et al. 1997; Reynolds & Guillaume 1998).

A study of a population from Cambridgeshire, U.K., found a higher release rate of juvenile bitterling from *U. pictorum* than from *A. cygnea* (Reynolds et al. 1997). However, the observed pattern did not account for the number of bitterling embryos that might have been ejected prematurely by mussels, as observed by Kondo et al. (1987) in a related species (*R. ocellatus ocellatus*). A study of mussel preference in the Czech Republic has confirmed that females avoid spawning in *A. cygnea*, but indicated no significant difference in preference for the other three species (Smith et al. 2000).

We extended this research with experiments to test for associations between host preference and offspring survival as determined by retention or ejection of embryos from mussels. We focused primarily on variation between mussel species, but we also tested for adaptive flexibility of individual bitterling. We also compared results from the area of recent sympatry in the U.K. with published results from an area of ancient contact in the Czech Republic to distinguish between the evolutionary lag and equilibrium hypotheses for the bitterling–mussel interaction.

## METHODS

### Study Sites

We collected individuals of the four mussel species, *U. pictorum*, *U. tumidus*, *A. anatina* and *A. cygnea*, from Reach Lode, a tributary of the River Cam, Cambridgeshire, U.K. at the point of confluence with Wicken Lode (N.G.R.: TL 545 696). They were collected before the bitterling reproductive season to ensure that they did not contain bitterling eggs. We collected the mussels by hand along the silt bottoms near to the banks, and transported them in aerated containers to outdoor plastic pools at the University of East Anglia. Fifty mussels were allocated to each pool (140 × 90 cm and 30 cm high) which was covered in a 10-cm layer of washed sand. The mussels in each pool were fed daily with 3 litres of a live algal suspension derived from an outdoor pool that had been seeded with *Chlorella vulgaris*.

We collected bitterling of both sexes from the same site, using high-frequency (600 Hz) pulsed DC Electrocatch WFC 12 electrofishing equipment. This equipment reduces recovery time and is less physiologically damaging than conventional equipment (Lamarque 1990; Bird & Cowx 1993). The fish were transported in aerated carriers to stock aquaria of volume 300 litres, containing 10 cm of gravel. The stock tanks were located in an indoor aquarium facility at the University of East Anglia. Forty fish were kept in each aquarium, which was continuously aerated by two under-gravel filters seeded with *Nitrosomonas* and *Nitrobacter* bacteria and a Tetra<sub>tec</sub> IN 1000 internal aquarium filter. The fish were fed a mixed diet of live *Daphnia pulex*, *Chaoborus* pupae, *Culex* larvae and pupae, chironomid larvae, frozen *D. pulex*, *Tubifex*, *Artemia salina*, dried protein mix and trout pellets.

The fish were acclimatized for 5 days at a temperature and photoperiod corresponding to natural conditions in the wild. The aquaria were illuminated by an Aqua-Glow 40-W fluorescent aquarium lamp with a 14:10 h light:dark regime and the water was kept at 12°C. After this period the bitterling were brought into reproductive condition by an increase in water temperature to 22°C, and an increase in the photoperiod to 16:8 h light:dark. To mimic the period of dawn and dusk, room lighting but not aquarium lighting was applied for 30 min prior to and after the photoperiod.

### Experiment 1: Host Preferences with Multiple Mussels

We did experiment 1 in May 1997 and again in May 1998. Each year, we used 15 mussels of each of the four species to test for a rank order of host preference by bitterling. We placed one individual of each species in one of four round sand-filled glass containers (10 cm diameter × 6 cm high) arranged 20 cm apart in a square formation within a 300-litre aquarium. The mussels were enclosed in perforated plastic bottles which enabled normal filtration of phytoplankton while preventing the bitterling from spawning. We then placed four bitterling in the aquarium. We focused on the behaviour of the

largest male and female, which were in reproductive condition, while the second pair of fish were used to help induce territorial behaviour by the dominant male.

When all four mussels were ventilating, we removed the bottles from the mussels and recorded the behaviour of the bitterling for 1 h. The minimum requirement was to ascertain which mussels the fish preferred, as shown by prespawning behaviour (Wiepkema 1961). We recorded the frequencies of the following behaviours.

(1) Male and female behaviours: mussel inspection (bitterling approach and face the top of a mussel), head-down posture (bitterling mouth is just above a mussel's exhalant siphon at about an angle of 45° or more).

(2) Male behaviours: skim (male dips forward over the top of a mussel, but no sperm is visible), sperm release (a white sperm cloud is visible), quivering (rapid lateral undulating movement of the body and fins in front of the female), leading (male orients itself towards the mussel in front of the female and quivers while swimming in the direction of the mussel).

(3) Female behaviours: failed spawning attempt (female misses the mussel siphon with ovipositor) and successful spawning (ovipositor momentarily rigid in exhalant siphon).

For each replicate ( $N=30$ ), we used new fish and mussels. The positions in which the mussel species were placed relative to each other were alternated among replicates.

## Experiment 2: Host Preferences with No Mussel Choice

We collected 40 individuals of each of the four mussel species in March 1999. We used eight aquaria (300 litres), bisected by a meshed partition that allowed water to flow, yielding 16 experimental tanks. We placed one mussel in a sand-filled glass container in the centre of the 150-litre tank and covered it with a perforated plastic bottle. We then added to the tank one male bitterling in full breeding coloration and one female that had a long transparent ovipositor.

The experiment began when the mussel opened its siphons and started ventilating, at which point we removed the cover and started the timer. We assessed host preference as the time until either a failed spawning attempt or a successful spawning. Observations were made until the bitterling pair had spawned twice in the mussel. We then removed the mussel and added a mussel of a different species until the pair had spawned twice in all four species of mussel. We alternated the sequence in which the mussels were presented among replicates, yielding a randomized complete block design. We obtained 27 replicates of this experiment, that is, 27 pairs of fish spawned in all four mussel species; however, we obtained only 22 and 24 spawnings in *U. tumidus* and *A. cygnea*, respectively. The mean lengths  $\pm$  SE of the mussels were: *U. pictorum*: 76.9  $\pm$  1.8 mm; *U. tumidus*: 71.9  $\pm$  2.6 mm; *A. anatina*: 81.0  $\pm$  3.1 mm; *A. cygnea*: 96.1  $\pm$  2.3 mm.

## Egg Deposition by Female Bitterling

We repeated the procedure for experiment 2 with a further 10 bitterling pairs and 10 mussels of each species. After the fish spawned, we froze the mussels and later dissected them to expose the gill lamellae and count the number of eggs laid in each gill demibranch. We recorded the position of the eggs in the gill lamellae, the distance of each egg from the exhalant siphon and the total length of the gills. The mean lengths  $\pm$  SE of the mussels were: *U. pictorum*: 81.3  $\pm$  2.8 mm; *U. tumidus*: 72.6  $\pm$  3.5 mm; *A. anatina*: 93.0  $\pm$  2.2 mm; *A. cygnea*: 86.7  $\pm$  4.5 mm.

## Mussel Host Quality in Terms of Egg Ejections

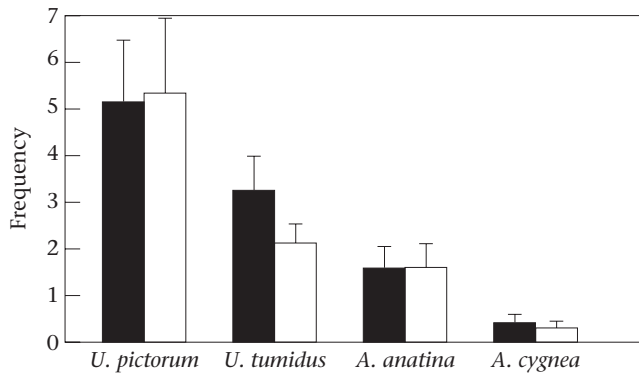
To assess the quality of each mussel host used in the second host preference experiment, we monitored bitterling egg mortality in the mussels that had received spawnings in experiment 2. We transferred the mussels to a plastic trough (75  $\times$  22 cm and 17 cm high) after two successful spawnings. The 84 troughs were arranged in 21 blocks of four following a Latin Square design. Each trough contained a 10-cm layer of washed sand and 10 litres of dechlorinated water with continuous aeration and mixing of the water. The mussels were kept at a constant temperature of 22°C with a 16:8 h light:dark regime throughout the experiment and were fed 1 litre of a live algal suspension derived from an outdoor pool that had been seeded with *Chlorella vulgaris* daily. During twice-daily observations of the troughs at 1000 and 2200 hours, we recorded the number of bitterling (embryos or larvae, live or dead) ejected from the mussels.

## Individual Preferences

We wished to determine whether bitterling that spawned most quickly into a mussel from the 'wrong' genus was nevertheless making the right decision. That is, suppose a female went against the majority preference and spawned most quickly into a mussel from a genus that was on average less preferred, and that tended to eject the most embryos. This spawning decision could still be adaptive if that particular mussel were less likely to eject its offspring than the alternative. We tested this possibility by analysing the data from the preference and ejection experiment described above to ask whether the preferences shown by individual fish corresponded to individual mussel ejection rates, regardless of the genus of the mussel.

## Ethical Note

After the experiments, all bitterling and mussels, with the exception of the mussels that were dissected, were returned to the study site. The removal of mussels would not have had an impact on the local population: in surveys of 10 m at the study site, 120 *U. pictorum*, 80 *A. anatina*, 45 *U. tumidus* and 120 *A. cygnea* were found after 2 h of sampling.



**Figure 1.** Mean  $\pm 1$  SE number of skims and sperm cloud releases by males/h and the mean number of failed spawning attempts and successful spawnings by females/h into four species of mussel.  $N=30$  in all cases. ■: Males; □: females.

### Statistical Analyses

To test host preferences and mussel host quality, we used a univariate general factorial model procedure with a randomized complete block design and two treatments (Sokal & Rohlf 1997). The data used for these tests, time until failed spawning attempts and successful spawnings, number of eggs laid and the number of eggs ejected, were log transformed. Multiple comparison procedures were carried out with the Tukey test.

## RESULTS

### Experiment 1: Host Preferences with Multiple Mussels

Male and female bitterling showed significant host preferences for *U. pictorum*, followed by *U. tumidus*, *A. anatina* and *A. cygnea*. This was shown by the number of times males skimmed over the mussels and released sperm clouds (Kruskal–Wallis test:  $H_3=18.55$ ,  $P<0.001$ ; Fig. 1), the number of failed spawning attempts and successful spawnings by females (Kruskal–Wallis test:  $H_3=22.91$ ,  $P<0.001$ ; Fig. 1) and the number of male quivers and attempts to lead females to mussels (Kruskal–Wallis test:  $H_3=16.61$ ,  $P<0.001$ ; Table 1). Significant differences were found between host species for host inspection rates by male and female bitterling (Kruskal–Wallis test: males:  $H_3=21.69$ ,  $P<0.001$ ; females:  $H_3=21.31$ ,  $P<0.001$ ; Table 1). Bitterling preferred *U. pictorum*, *U. tumidus* and *A. anatina* over *A. cygnea* (all  $P$  values  $<0.05$ ).

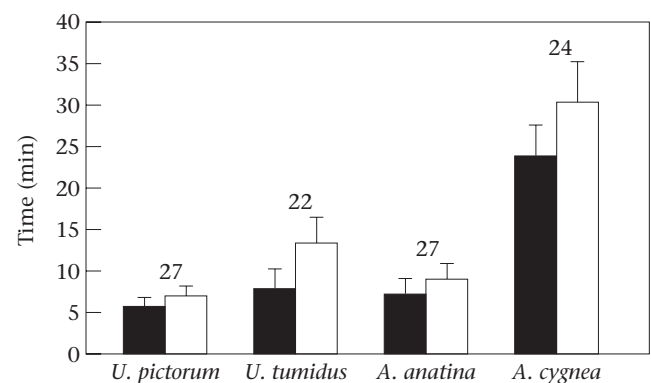
### Experiment 2: Host Preferences with No Mussel Choice

There was a significant difference between the four mussel species in the time bitterling pairs took for a failed spawning attempt (GLM:  $F_{3,96}=14.79$ ,  $P<0.001$ ; Fig. 2) and for a successful spawning (GLM:  $F_{3,96}=13.22$ ,  $P<0.001$ ; Fig. 2). Post hoc analyses revealed that the difference was between *A. cygnea* and the other three mussel species for both failed and successful spawning

**Table 1.** Differences in the mean hourly rate of breeding behaviour of male and female bitterling in the presence of four mussel host species

Mussel species	Quivers and leads by males	Head-downs and inspections by males	Head-downs and inspections by females
<i>U. pictorum</i>	$6.1 \pm 1.7^a$	$17.8 \pm 3.5^a$	$6.4 \pm 1.3^a$
<i>U. tumidus</i>	$3.1 \pm 1.0^a$	$14.6 \pm 3.2^a$	$4.9 \pm 0.8^a$
<i>A. anatina</i>	$1.9 \pm 0.6^{ab}$	$9.4 \pm 2.1^a$	$3.7 \pm 0.8^a$
<i>A. cygnea</i>	$0.4 \pm 0.2^b$	$3.0 \pm 0.7^b$	$0.8 \pm 0.3^b$

Mean  $\pm$  SE number of behaviours/h.  $N=30$  in all cases. Significant differences are shown by different letters: species shown as ab are not significantly different from either a or b.



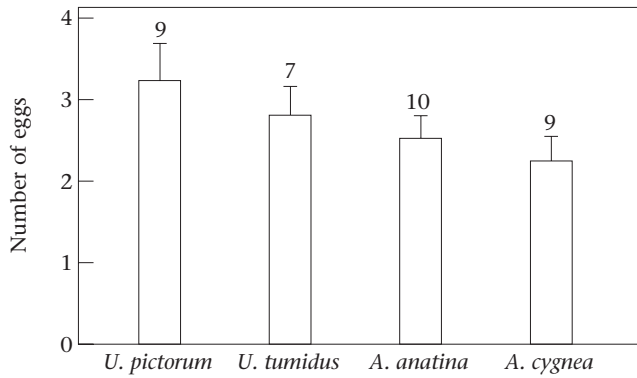
**Figure 2.** Mean  $\pm 1$  SE time taken until females performed spawning behaviour into the four species of mussel when not given a choice. Numbers above bars refer to numbers of replicates. ■: Failed spawning attempt; □: successful spawning.

attempts (Tukey HSD: failed spawnings: *U. pictorum*–*A. cygnea*:  $q_{97}=7.10$ ; *U. tumidus*–*A. cygnea*:  $q_{97}=5.30$ ; *A. anatina*–*A. cygnea*:  $q_{97}=6.29$ ; successful spawnings: *U. pictorum*–*A. cygnea*:  $q_{96}=6.16$ ; *U. tumidus*–*A. cygnea*:  $q_{96}=4.28$ ; *A. anatina*–*A. cygnea*:  $q_{96}=5.76$ ; all  $P$ s  $<0.001$ ). The sequence in which the mussel hosts were presented had no significant effect on the time taken for a failed spawning attempt or a successful spawning (GLM: failed:  $F_{3,96}=0.82$ ,  $P=0.49$ ; successful:  $F_{3,96}=0.98$ ,  $P=0.41$ ).

### Egg Deposition by Female Bitterling

The number of eggs laid differed significantly between the mussel species (GLM:  $F_{3,32}=4.12$ ,  $P=0.024$ ; Fig. 3). More eggs were laid in *U. pictorum* than in *A. cygnea* (Tukey HSD:  $q_{32}=4.38$ ,  $P=0.03$ ). The spawning sequence had no effect on the number of eggs laid in each host (GLM:  $F_{3,32}=0.56$ ,  $P=0.66$ ).

The distance of the eggs from the exhalant siphon as a proportion of the total length of the gills was significantly greater in *U. pictorum* and *U. tumidus* than in both of the *Anodonta* species (proportion of gill length  $\pm$  SE: *U. pictorum*:  $0.37 \pm 0.02$ ; *U. tumidus*:  $0.41 \pm 0.04$ ; *A. anatina*:  $0.28 \pm 0.02$ ; *A. cygnea*:  $0.29 \pm 0.02$ ; GLM:  $F_{3,30}=5.99$ ,  $P=0.041$ ). This result was not due to a longer gill in



**Figure 3.** Mean+1 SE number of eggs laid in the four species of mussel. Numbers above bars refer to numbers of mussels.

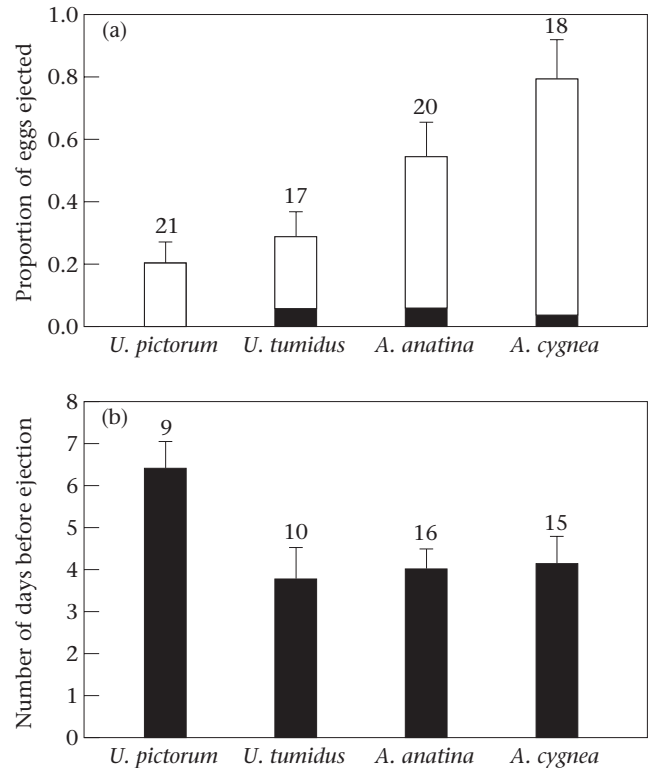
*Anodonta* spp., as there was no significant difference in total gill length between the mussel species (total length: *U. pictorum*:  $4.73 \pm 0.21$  cm; *U. tumidus*:  $4.63 \pm 0.37$  cm, *A. anatina*:  $5.39 \pm 0.15$  cm; *A. cygnea*:  $4.72 \pm 0.31$  cm; ANOVA:  $F_{3,32}=2.18$ ,  $P=0.112$ ).

### Mussel Host Quality in Terms of Egg Ejections

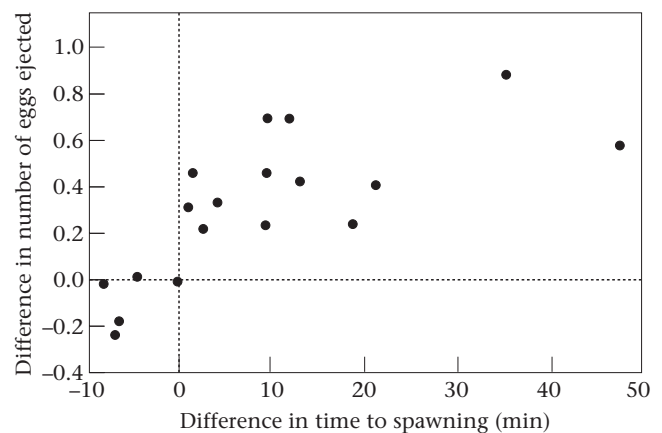
There was a significant difference between mussel species in the proportion of eggs ejected after spawning (GLM:  $F_{3,73}=8.44$ ,  $P<0.001$ ; Fig. 4a). Each of the *Anodonta* species ejected significantly more eggs than each of the *Unio* species (Tukey HSD: *U. pictorum*–*A. anatina*:  $q_{73}=3.81$ ; *U. pictorum*–*A. cygnea*:  $q_{73}=4.61$ ; *U. tumidus*–*A. anatina*:  $q_{73}=3.93$ ; *U. tumidus*–*A. cygnea*:  $q_{73}=4.23$ ; *A. anatina*–*A. cygnea*:  $q_{73}=3.71$ ; all  $P_s < 0.05$ ). This was also the case when the number of eggs ejected included the eggs that were ejected at the time of spawning (GLM:  $F_{3,73}=8.7$ ,  $P<0.001$ ; Tukey HSD: *U. pictorum*–*A. anatina*:  $q_{73}=3.73$ ,  $P<0.005$ ; *U. pictorum*–*A. cygnea*:  $q_{73}=4.85$ ,  $P<0.001$ ; *U. tumidus*–*A. anatina*:  $q_{73}=3.13$ ,  $P<0.05$ ; *U. tumidus*–*A. cygnea*:  $q_{73}=3.88$ ,  $P<0.001$ ; Fig. 4a). Over three-quarters of eggs laid in *A. cygnea* were ejected, whereas only 20% were ejected from *U. pictorum*, which also took longer to eject eggs than the other three species, although this was not quite significant (GLM:  $F_{3,46}=2.78$ ,  $P=0.077$ ; Fig. 4b). The proportion of eggs ejected by each mussel was not affected by the spawning sequence. However, there was a significant difference between bitterling pairs in the proportion of eggs that were ejected (GLM:  $F_{19,57}=2.30$ ,  $P=0.013$ ). These differences were not significantly related to the lengths or weights of the fish (data not shown).

A significant positive correlation was found between the time taken for an individual female to spawn in a host species (host preference) and the number of eggs that were ejected from that mussel (host performance) (Spearman rank correlation:  $r_s=0.63$ ,  $N=69$ ,  $P<0.001$ ).

We have concentrated on bitterling preferences among mussel species but we also examined host preference behaviour for individual mussels. We tested whether differences between individual mussels in the time taken for successful spawnings were correlated with



**Figure 4.** (a) Mean+1 SE proportion of eggs laid in each mussel species that were ejected during (■) and after (□) spawning. (b) Mean+1 SE number of days until egg ejection in the four species of mussel. Numbers above bars refer to numbers of mussels.



**Figure 5.** Relationship between the host preference of individual females for the two mussel genera. Each point shows the mean time taken for a successful spawning in *Anodonta* species minus the mean time taken for a successful spawning in *Unio* species for each female bitterling, and the mean number of eggs ejected in *Anodonta* species minus the mean number of eggs ejected in *Unio* species.

differences between mussels in egg rejections. Figure 5 shows that when individual females discriminated more strongly between *Anodonta* and *Unio*, this corresponded to greater differences between the genera in egg ejection rates (Spearman rank correlation:  $r_s=0.80$ ,  $N=18$ ,  $P<0.001$ ).

## DISCUSSION

Both male and female bitterling discriminated between mussel species, as shown by which mussels they chose, the time until spawning and the number of eggs deposited. These individual preferences appeared to be adaptive as they were positively correlated with host quality in terms of acceptance of eggs.

*Unio pictorum* was the preferred host mussel species (Figs 1, 2, 3): it ejected the fewest eggs on average (Fig. 4a) and took the longest time to eject them (Fig. 4b). Conversely, the least favoured host *A. cygnea* ejected the greatest proportion of eggs (Fig. 4a). If the bitterling chose the mussel species randomly the probability of choosing them in a sequence of declining quality was  $1/24$  (number of mussels decreases by 1 after each choice:  $\frac{1}{4} \times \frac{1}{3} \times \frac{1}{2} \times \frac{1}{1} = \frac{1}{24}$ ). Therefore, we suggest that bitterling host preferences may have evolved in response to natural selection imposed by mussel egg acceptance rates. The adaptive nature of the host preferences that we found in our laboratory experiments matches the pattern of natural host productivity at our study site. Reynolds et al. (1997) found that *U. pictorum* produced the most bitterling larvae and *A. cygnea* the fewest. While the majority of our results focus on mean population choices by bitterling and responses by mussels, our experiments also enabled us to test for adaptive choice by individual females. We found that some female bitterling chose *Anodonta* over *Unio* species and, although this went against the population average, their decisions proved to be adaptive as they selected a mussel with the lowest ejection rate, regardless of species.

We have found that ventilation rates of *U. pictorum* are reduced by about two-thirds when the mussels receive moderate numbers of eggs (unpublished data). Why, then do *U. pictorum* usually accept bitterling eggs and larvae? Below we interpret our findings with respect to the two main hypotheses that explain the evolution of adaptations in host–parasite systems.

### Arms Race–Evolutionary Lag Hypothesis

A previously unparasitized lineage of mussels could either lack adequate genetic variation for egg ejection behaviour or the behaviour might not have had time to spread in the population (Rothstein 1975a, b; Dawkins & Krebs 1979; Davies & Brooke 1989a, b; Moksnes et al. 1990). The best test of the lag hypothesis is a comparison between parasite–host systems of different ages, as has been shown for avian brood parasites (Soler & Møller 1990; Briskie et al. 1992). The earliest date that the bitterling and mussels studied here have been in sympatry is the beginning of the 1900s when bitterling are thought to have been introduced from continental Europe (Hardy 1954; Wheeler & Maitland 1973; Maitland & Campbell 1992). This system can be compared with one in the Czech Republic where the ranges of all four mussel species have an ancient history of sympatry with that of the bitterling (Ellis 1978; Lelek 1980). In both populations there is strong avoidance of *A. cygnea* by bitterling, and low survival of eggs in that species (Smith

et al. 2000). The U.K. population of *A. cygnea* has been exposed to bitterling for a maximum of 20 mussel generations as the youngest recorded gravid mussels were aged 5 years (Heard 1975). Given the low prevalence of parasitism in this species, we feel it is unlikely that *A. cygnea* has evolved such strong ejection behaviour in such a short time. One could salvage the arms race–evolutionary lag hypothesis if *A. cygnea* that separated from the mainland at the Pleistocene glaciation were ejectors and their descendants retained this trait in the absence of selection. To test this we need to examine ejection behaviours in mussels from areas known never to have supported bitterling. The retention of relic behaviours such as egg rejection in the absence of brood parasitism has been found in a number of Caribbean and Californian cowbird–host populations (reviewed in Rothstein 2001).

### Evolutionary Equilibrium Hypothesis

The evolutionary equilibrium hypothesis suggests an adaptive explanation for the acceptance of parasitic eggs, whereby the costs of egg ejection exceed its benefits (Zahavi 1979; Rohwer & Spaw 1988; Brooker & Brooker 1990; Petit 1991; Lotem et al. 1992; Lotem & Nakamura 1998; Røskoft & Moksnes 1998). Our finding that eggs are lodged more deeply into the gills of *Unio* species than *Anodonta* species suggests a possible physical limitation on the ability of *Unio* species to dislodge eggs. However, this cannot be the full explanation for differences in mussel responses, since the distances of egg deposition into the gills did not differ within genera. It is also possible that mussel species are constrained by the risk of ejecting their own larvae (glochidia) from their gills when ejecting bitterling. We have occasionally seen premature glochidia ejection during bitterling spawning. *Unio* species might be more at risk (and therefore more likely to accept eggs) because their period of glochidia brooding coincides with a larger portion of the bitterling spawning season.

### Origins of Ejection

Possible evolutionary origins of host egg ejection and gill evacuation by mussels may include the expulsion of pseudofaeces (Jørgensen 1990) and the release of glochidia (Tankersley 1996). Thus, while the use of this behaviour may be an antiparasite adaptation to the presence of bitterling eggs, it could also be a generalized behaviour for ejecting objects in their gills. The differences between mussel species in egg ejection behaviour may be based on differences in host physiology. For example, the rank order of ventilation rates of the four species matches their order of ejection (Mills & Reynolds 2002).

In conclusion, we have shown that bitterling show adaptive and flexible host preferences among the four species of mussels available. Our findings do not suggest an arms race–evolutionary lag in responses of mussels.

Rather, these may represent an evolutionary equilibrium with ejection costs. Alternatively, the mussels' behaviours might not have been shaped by coevolutionary interactions with bitterling, but may be by-products of differences between species in physiology. Studies of mussel responses in populations that have never been exposed to bitterling are needed to test this possibility.

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