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Parasites can cause selection against migrants following dispersal between environments

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Summary

1. The potential for selection against migrants to promote population divergence and speciation is well established in theory, yet there has been relatively little empirical work that has explicitly considered selection against migrants as a form of reproductive barrier, and its importance in the accumulation of reproductive isolation between populations has been overlooked until recently.

2. Parasites often differ between environments and can be an important source of selection on hosts, yet their contribution to population divergence in general, and selection against migrants in particular, is poorly understood.

3. Selection against migrants might be reduced if organisms escape parasitism when they disperse (natural enemy release). Alternatively, parasites could increase selection against migrants if, when they disperse, organisms encounter parasites to which they are poorly adapted.

4. Here we test experimentally the contribution that parasites could make to selection against migrants in the adaptive radiation of three-spined sticklebacks. These fish have repeatedly colonized freshwater environments from marine ones, and this has repeatedly lead to rapid speciation.

5. We use transplant experiments of lab-raised fish to simulate dispersal, and antihelminthic treatment to show that ancestral-type marine sticklebacks contract higher burdens of novel parasites when introduced to freshwater, than in saltwater, and suffer a growth cost as a direct result.

6. Susceptibility to parasites and their detrimental effect is less in derived, freshwater fish from evolutionarily young populations, possibly as a result of selection for resistance.

7. Our results support a role for parasites in selection against migrants and population diversification. They do not support the hypothesis of 'natural enemy release'.

Key-words: adaptive radiation, ecological speciation, enclosure experiment, *Gasterosteus aculeatus*, host-parasite interaction, immigrant inviability, natural enemy release, reproductive isolation

Introduction

Reproductive isolation between diverging populations is central to speciation. Indeed, from the perspective of the biological species concept the evolution of reproductive isolating barriers *is* speciation (Mayr 1942; Coyne & Orr 2004). Despite more than half a century of work on isolating barriers (Dobzhansky 1951), it has been recently pointed out that there is an important form of barrier which has been empirically rather overlooked (Hendry 2004; Nosil, Vines & Funk 2005). Reproductive isolation has been thought to occur most commonly because populations become spatially isolated or otherwise fail to meet during the mating season, or because they do meet but fail to recognize one another, or because the hybrid offspring that they produce are inferior in some way. However, even if none of these conditions apply, another process could still contribute to reproductive isolation. Speciation is often associated with colonization of new environments from an ancestral stock (Darwin 1859). As local adaptation begins to occur in the descendant population, the relative fitness of migrants from the ancestral population may decline, because of inferior survival or fecundity in the new

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environment (Hendry 2004; Nosil, Vines & Funk 2005). Such selection against migrants or 'immigrant inviability' could lead to a constriction of gene flow between the diverging populations and hence be an important source of reproductive isolation between them. This idea was established in theory decades ago (Balkau & Feldman 1973; Dickinson & Antonovics 1973), and has continued to stimulate theoretical work. However, it has made little impact on field studies of speciation until recently (Hendry 2004; Nosil, Vines & Funk 2005).

The role of natural enemies in promoting divergence has become a topic of substantial interest in recent years (Vamosi 2005; Nosil & Crespi 2006), but such work has concentrated almost exclusively on the effect of predators, while the role of parasites is poorly understood. Here we investigate experimentally the possibility that parasites can cause selection against migrants in the adaptive radiation of three-spined sticklebacks (*Gasterosteus aculeatus* L.).

The immediate ecological importance of natural enemies (including parasites and herbivores), when colonizations first take place, have been well explored in the context of anthropogenic invasions (Parker, Burkepile & Hay 2006; Borer *et al.* 2007), especially by plants. We are more interested in the evolutionary consequences that natural enemies might have in the context of natural colonizations. Nevertheless, the ecological literature is informative about the kinds of outcome that might be expected. It is clear from this literature that changes in parasitism (including pathogens and diseases) could reduce selection against migrants, if migrants escape from their worst parasites when they disperse from their ancestral environment (Mitchell & Power 2003; Torchin *et al.* 2003). So-called 'natural enemy release' (Keane & Crawley 2002) is often suggested as a factor favouring the success of anthropogenic invasions. However, more recently the generality of the hypothesis has been questioned (Colautti *et al.* 2004; Parker, Burkepile & Hay 2006; Hawkes 2007), and very little appears to be known about whether natural enemy release alters the success of migrants between established natural populations.

The possibility that parasites in new environments could increase selection against migrants from ancestral populations is suggested by three lines of evidence. Firstly, meta-analyses of ecological studies of invasions suggest that 'biotic resistance', in which migrants actually encounter natural enemies that are worse than in their native range, may be more common than natural enemy release (Parker, Burkepile & Hay 2006), although conclusions about this are mixed (Maron & Vila 2001; Engelkes *et al.* 2008). These studies are biased towards the examination of the impact of herbivores (and sometimes pathogens) on invasions by plants, and there has been less experimental work on the role of natural enemies in anthropogenic invasions of animals, and none that we are aware of that have looked at the effect of parasites on selection against migrants following natural colonizations by animals. Secondly, experimental studies of local adaptation show that hosts can be more susceptible to allopatric than sympatric parasites, especially where gene flow between host populations is higher than that between parasite populations (Hoeksema & Forde 2008). Thirdly, and more graphically, there are a number of well known examples in which parasitic organisms have invaded new areas and devastated populations of native hosts, e.g. avian pox virus and malaria (*Plasmodium relictum*) in Hawaiian endemic birds and rinderpest virus in African ungulates (Lafferty *et al.* 2005). All of these lines of evidence suggest that when hosts migrate to a different environment the parasites there may reduce their fitness, causing selection against migrants and contributing to reproductive isolation between migrants and residents of the new environment. As far as we are aware there has been no previous experimental test of this hypothesis.

The only way to establish experimentally whether parasites contribute to selection against migrants is to artificially introduce organisms into a novel environment within an evolutionarily realistic context (Parker & Gilbert 2007). This is seldom feasible with animals because of ethical and conservation concerns, but may be possible with organisms that often perform natural colonizations. Three-spined sticklebacks (hereafter 'sticklebacks') are a naturally invasive, small, temperate fish. They have large, evolutionarily conserved populations in the northern Pacific and Atlantic oceans and adjoining seas that have remained more or less unchanged for several million years (Bell & Foster 1994). These marine sticklebacks provide a good approximation to the ancestral state of sticklebacks now found in freshwater (Bell & Foster 1994). Current freshwater populations were established by many independent colonizations of rivers and lakes by these marine sticklebacks (Schluter 2000; Makinen, Cano & Merila 2006), and thus at some point in the past marine fish must have migrated to freshwater and remained there to establish these populations. However, in many places this phenomenon, which was obviously once common, no longer occurs: anadromous marine fish commonly continue to migrate to freshwater to breed, but they return to the sea afterwards, and are often completely reproductively isolated from the phenotypically very different freshwater fish (McKinnon & Rundle 2002). What can have lead to such reproductive isolation between populations for which there has been no obvious history of allopatry and which now breed alongside each other in the same microhabitats at the same time of year, and can produce fully viable hybrid offspring when artificially crossed? One possibility is that, as the descendants of early colonists of freshwater became adapted to their new environment, later migrants from the sea became relatively less fit. Here we test the idea that parasites may have contributed to this selection against migrants in the adaptive radiation of sticklebacks.

The parasites of sticklebacks are diverse, but relatively well known (Barber 2007), and can differ substantially between host populations (Wegner, Reusch & Kalbe 2003; MacColl 2009). There are many parasite species that occur in freshwater that cannot be transmitted in the sea because intermediate hosts (e.g. freshwater insects and planktonic crustacea) do not occur there. This suggests that changes in parasite exposure could influence the colonization success of marine sticklebacks dispersing to freshwater.

We conducted two experiments in which we sought to simulate dispersal to freshwater by marine sticklebacks, and to examine the impacts of parasite infections on this process, in comparison to their impact on native freshwater fish. In the first experiment lab-raised juvenile Atlantic marine sticklebacks were introduced into enclosures in both tidal marine and freshwater environments on the island of North Uist, Scotland. Their growth (as a measure of fitness) and the parasites they contracted were compared between environments and to lab-raised juvenile freshwater fish from North Uist introduced into enclosures in freshwater. In order that we might ascribe causation of any growth differences to parasite infection, we treated some fish with antihelminthics to reduce their parasite burdens (Gulland *et al.* 1993). In the second experiment, sticklebacks of both a Pacific marine and Canadian freshwater origin were introduced into enclosures in a pond in British Columbia, Canada with few natural parasites. In this experiment we attempted to experimentally increase the parasite burden of some fish by adding to some enclosures the intermediate hosts of an important freshwater stickleback parasite. The second experiment allowed us to expose the freshwater fish to parasites from a lake other than their own, and therefore to remove the possibility that any observed differences between marine and freshwater fish might be a result of differences in local adaptation of host or parasite.

Materials and methods

EXPERIMENT 1, SCOTLAND

The experiment was carried out from April until September 2006 under licence from the UK Home Office, with the approval of the University of Nottingham Ethical Review Committee and with the permission of the Scottish Executive. Seven families each of freshwater fish and marine (Atlantic) fish were established in the lab in Nottingham by artificial crossing of wild parents caught on the island of North Uist, Scottish Outer Hebrides. Freshwater fish were reared from eggs (clutches of 20) in freshwater only. Clutches of marine fish (40 eggs) were split and reared in either freshwater ('fresh-reared') or 20‰ marine salt ('salt-reared'). The experiment did not have a factorial design, because we were not interested in the performance of freshwater fish reared in a salt environment. Instead, the experiment was designed to allow us to explore the effect of rearing environment on marine fish, and the consequences of population provenance (marine or freshwater) for performance in freshwater.

Parents of freshwater fish came from Loch na Buaile (57°39′N 7°14′W, UK, Ordnance survey grid reference NF900738), a small (*c.* 4 ha), shallow (< 3 m) loch whose only fish species are sticklebacks and occasional eels (*Anguilla anguilla* L.). Parents of marine fish were anadromous sticklebacks caught at the mouth of Loch an Struth Mhoir (NF905696) a brackish loch that is regularly flooded by the high tide. Mature eggs were stripped from gravid females and fertilized *in vitro* using minced testes from euthanized males from the same population. Each batch of 20 eggs was hatched in one half of a 100 L aquarium divided by a very fine mesh screen to allow water circulation. Fry were fed with infusoria (*Colpidium* sp.) for the first 2 days, then on freshly hatched brine shrimp (*Artemia* sp.) nauplii twice a day. At approximately 1 month, chopped Chironomid larvae ('bloodworm', defrosted from frozen) were introduced to the diet. At approximately 3 months of age fish were transported to North Uist for introduction to enclosures in Loch na Buaile and Fairy Knoll lagoon (NF889726), a shallow (< 3 m) tidal lagoon close to Loch an Struth Mhoir, but with a sediment bottom ideal for the siting of enclosures. The salinity at Fairy Knoll fluctuates between approximately 15‰ and 25‰, depending on the state of the tide (A. D. C. MacColl, unpublished data). Freshwater fish were only introduced to enclosures in freshwater (Loch na Buaile), whereas fresh-reared marine fish were introduced into freshwater enclosures and salt-reared marine fish were introduced into enclosures in salt water (Fairy Knoll) (Fig. 1).

Enclosures were 1 m^3 'boxes' (1·2 × 1·2 × 0·7 m) constructed of 1 mm fly screen mesh. All sides were closed and access into each enclosure was only possible through a zip on one side. The experiment was planned as a split plot design. Enclosures (plots) were arranged in six groups (blocks) of four (in Loch na Buaile) or two (in Fairy Knoll) along the shore at 10–100 m intervals. Enclosures were situated in water 70–80 cm deep (Loch na Buaile) or 30–150 cm deep (Fairy Knoll, dependent on the tide), 2–10 m from the shore, and were supported by wooden stakes hammered into the silt substrate. Approximately 10 L of substrate were distributed across the bottom of each enclosure 1 week before any fish were added. In each group, one enclosure was allocated at random to each treatment combination. In freshwater the treatments were 'provenance' of the fish that were placed in that enclosure (marine or freshwater) and 'antihelminthic treatment' (treatment or control, see below). In salt water the only treatment was antihelminthic treatment or control. Thirty fish of each provenance and rearing environment had their standard length (mm) recorded to the nearest half millimetre immediately before addition to the enclosures. Six haphazardly selected fish of the appropriate provenance were added to each enclosure.

After 26 days, minnow traps ('Gee' traps; Tackle Factory, Fillimore, NY, USA) were put into each enclosure. All fish captured overnight had approximately 0·5 mm of their first dorsal spine clipped. Clipped fish from enclosures assigned to the antihelminthic treatment were contained for 2 h in 6 L of loch water containing 2 mg mL^{-1} praziquantel ('Equitape', Fort Dodge Animal Health) and 2 mg mL^{-1} fenbendazole ('Panacur', Intervet). DMSO was used to facilitate the solution of both drugs in water, so clipped fish from enclosures assigned to the antihelminthic control were contained for 2 h in

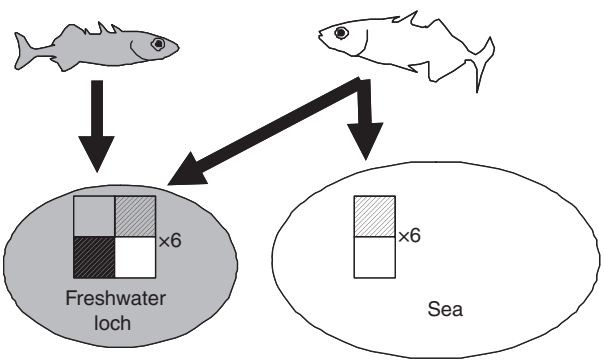

Fig. 1. The design of experiment 1 (Scotland). Fish of freshwater (shaded) and marine (unshaded) provenance were reared in the lab. Freshwater fish were introduced into enclosures (squares in the figure) in freshwater only. Marine fish were introduced into enclosures in both freshwater and the sea. Some fish of each provenance were treated with antihelminthic (hatched squares), others were left untreated. Groups of enclosures (experimental blocks) were replicated six times in each environment.

6 L of loch water containing the appropriate amount of DMSO (2 mL). Thus at the end of the experiment there were three groups of fish: 'clipped' antihelminthic treated fish, clipped antihelminthic control fish and a third unclipped control group for the capture and clipping intervention. Twenty-one days after antihelminthic treatment was administered, the experiment was terminated. Following removal of fish by trapping for two nights, all enclosures were removed from the loch and searched exhaustively by passing all substrate though a 4 mm sieve. Captured fish were euthanized by overdose of MS222, their standard length was measured and they were individually stored in 70% ethanol until they could be dissected.

The body surface, fins, eyes, opercular cavities, gill rakers, body cavities and all internal organs of recovered fish were dissected under a binocular microscope and searched for parasites. The abundance of all macroparasites and the occurence of cysts of *Glugea anomala* (Microsporidia) were recorded. Data were analysed in GenStat 10 (VSN International Ltd, Hemel Hempstead, UK). Log-linear models were used to analyse prevalence data. Parasite abundances and final length data were analysed with linear mixed models (LMMs) with appropriate fitted terms (see Results). Block, and enclosure nested within block, were fitted as random effects, in accordance with the split plot design of the experiment (Galwey 2006). Negative binomial errors and a logarithm link function were used when analysing data on parasite abundances. Normal errors and an identity link function were used when analysing variation in final length. Denominator degrees of freedom in LMMs were calculated in GenStat using the Kenward-Rogers algorithm that adjusts for the inclusion of random terms in the model.

EXPERIMENT 2, CANADA

The experiment was carried out from March to August 2007 in British Columbia, Canada in accordance with Canadian Animal Care legislation and with the approval of the University of Nottingham Ethical Review Committee. Six families of freshwater fish and seven families of marine fish (Pacific) were raised to approximately 10 weeks of age in aquariums at the University of British Columbia (UBC), Vancouver using the same methods as those in experiment 1. Parents of freshwater fish came from the benthic population in Priest lake, Texada island (49°45′N 124°34′W). Parents of marine fish were anadromous sticklebacks from a breeding population in Oyster lagoon (49°37′N 124°02′W), a brackish water body connected to the sea at high tides.

Ten fish of each provenance, haphazardly selected from the different families, were introduced into each of 20 enclosures placed around the margin of a 23 × 23 m experimental pond on the UBC campus (Schluter 1994). Half of these enclosures were assigned at random to a parasite supplementation treatment (see below) and the rest served as controls. All fish were measured before being placed in their enclosure to give a mean 'initial length' for fish of each provenance in each enclosure. Enclosures were constructed of 1 mm fly screen mesh stapled to a wooden frame (75 cm wide × 145 cm long), dug into the substrate of the sloping sides of the pond. Water depth in each enclosure varied between about 16 and 64 cm deep. The amount of water enclosed was approximately 430 L. To seed enclosures with food, zooplankton (1500 mL of *c*. 3 organisms per mL, mainly copepods, *Daphnia*, ostracods and *Chaoborus*) were added to each enclosure on the same day that the fish were added, and twice more at 10-day intervals. This zooplankton was collected by pelagic tows of Paxton lake, Texada island.

Parasite transmission is generally low in the ponds (A. D. C. MacColl, unpublished data). Therefore we attempted to increase parasite burdens of half of the enclosures by the addition of snails (*Stagnicola* sp.) infected with the parasite *Diplostomum scudderi* Olivier. *Stagnicola* sp. snails are the intermediate host of this trematode which commonly infects the eyes of sticklebacks in natural freshwater populations in British Columbia (MacColl 2009). Snails with patent infections emit *D. scudderi* cercaria that infect sticklebacks by penetrating the skin and migrating through the host tissue to the eyes. Individual infected *Stagnicola* are capable of shedding thousands of *D. scudderi* cercaria (MacColl, personal observation). *Stagnicola* spp. are freshwater snails and there is no known marine intermediate host of *D. scudderi*.

Snails were collected manually from Paxton lake and returned to UBC. *Stagnicola* were screened for *D. scudderi* by exposing them to bright light in a change of water. Snails that emitted *D. scudderi* cercaria were recorded as infected. Snails that did not emit cercaria were screened a further two times at 24-h intervals before being recorded as uninfected. Two weeks after the sticklebacks were placed in the enclosures, two infected *Stagnicola* were added to each of the ten enclosures assigned to the parasite supplementation treatment and two uninfected snails were added to each of the remaining control enclosures. After a further 2 weeks three more infected or uninfected snails were added to each infected or uninfected enclosure respectively.

The experiment was terminated 53 days after the fish were placed in the enclosures. Fish were trapped out of the enclosures for two nights and then the pond was drained to below the level of the enclosures, which were searched exhaustively for any remaining fish. Freshwater and marine fish were separated on the basis of their very divergent external morphologies (Marchinko & Schluter 2007). The standard length of all fish was recorded. Fish were euthanized and stored in 70% ethanol until they could be dissected. Both eyes of each fish were dissected and all *D. scudderi* were recorded.

Mean initial length of the fish in each provenance by enclosure combination was subtracted from the final length of each fish to give an estimate of growth for each fish. 'Mean growth' of each provenance by enclosure combination was then calculated. Data were analysed as before, except that enclosure and enclosure × provenance were fitted as random effects in LMMs.

Results

EXPERIMENT 1, SCOTLAND

General outcome

Basic experimental parameters are shown in Table 1, including the number of fish treated, the number recovered and their mean length at the beginning and end of the experiment. There were no differences between clipped and unclipped controls in either growth [clipped = 3·2 ± 0·73 mm (mean ± SE), unclipped = 3·0 ± 0·28 mm, LMM, Wald $F_{1,61}$ = 0·31, P = 0·58, normal error distribution and identity link] or parasite abundance (clipped = 2·4 ± 0·72 diplostomula, unclipped = 2·2 ± 0·20 diplostomula, LMM, Wald $F_{1,61}$ = 0·9, P = 0·35, negative binomial error distribution and logarithm link function), and these fish were combined for subsequent analyses. A storm late in the experiment resulted in small holes in some enclosures, especially at Fairy Knoll, where it is likely that some fish escaped (Table 1). This made it impossible to analyse survival in the different treatments. Marine fish that had been reared in saltwater in the lab

Table 1. Basic experimental parameters for experiment 1 (Scotland), including the length of fish at the beginning and end of the experiment, the number of fish treated and the number recovered in different treatment classes. A storm caused damage to enclosures late in the experiment and some fish escaped. This prevented the use of these data to estimate survival

Parameter	Freshwater fish	Fresh-reared marine fish	Salt-reared marine fish
Mean length at start, mm (mean ± SE)	19·9 ± 0·42	17·4 ± 0·27	19·0 ± 0·32
Mean length at end, mm (mean ± SE)	23·8 ± 0·38	21·9 ± 0·35	22·8 ± 0·91
Original number of fish	72	72	72
No. antihelminthic treated	15	11	14
No. clipped controls	16	6	11
Total no. fish recovered	41	38	13
No. antihelminthic treated fish recovered	8	6	4
No. clipped control fish recovered	5	3	2

were longer than fresh-reared marines at the start of the enclosure experiment ($t_{58} = 3{\cdot}77$, $P < 0{\cdot}001$), but there was no difference in length by the end ($t_{49} = 1{\cdot}14$, $P = 0{\cdot}26$). This suggests that growth rates of marine fish were, on average, higher in freshwater.

Differences in parasitism between rearing environments

Among sticklebacks of marine provenance, *G. anomala* was found in six salt-reared fish from Fairy Knoll, but in none of the fresh-reared fish from Loch na Buaile (log-linear model, $\chi^2_1 = 17{\cdot}8$, $P < 0{\cdot}001$). The only other parasites recovered from salt-reared marine fish were one ectoparasitic *Caligus* sp., one intestinal trematode (*Podocotyle* sp.) and three subcutaneous trematode metacercariae ('blackspot'), each infecting a single fish from Fairy Knoll. None of these parasites were found in fresh-reared fish.

Diplostomum gasterostei Williams and *Tylodelphys clavata* von Nordmann were the commonest parasites of the fish reared in Loch na Buaile. These closely related trematode parasites are of a similar size and appearance and both infect the eyes (retina and humour) of sticklebacks. Larval stages of these species are difficult to separate in preserved material, and as a result we recorded them jointly as 'diplostomula'. Our data, and several surveys of wild caught sticklebacks dissected fresh, suggest that the majority of diplostomula from Loch na Buaile are *D. gasterostei* (A. D. C. MacColl, unpublished data). Diplostomula did not occur in salt-reared marine fish, and there were significant differences between rearing environments in both their prevalence (log-linear model, $\chi^2_1 = 30{\cdot}0$, $P < 0{\cdot}001$) and abundance (Table 2).

The only other parasites of fresh-reared marine fish were larval cestodes found in the body cavity (log-linear model for the difference in prevalence between rearing environments, $\chi^2_1 = 2{\cdot}5$, $P = 0{\cdot}12$). The cestodes were probably *Diphyllobothrium* spp., since these are the only common cestodes in wild caught sticklebacks from Loch na Buaile. *Diphyllobothrium* spp. that infect sticklebacks (*D. norvegicum* Vik and *D. dendriticum* Nitzsch), as well as *Diplostomum gasterostei* and *T. clavata* all have intermediate hosts that are confined to freshwater, and the parasites are not known to be transmitted in brackish or marine systems (Bykhovskaya-Pavlovskaya *et al.* 1964). None of these parasites were found in the salt-reared marine fish.

Differences in parasitism between provenances

Among the fish reared in freshwater, there was a significant difference in the prevalence of larval cestodes between marine (prevalence = 0·1) and freshwater fish (prevalence = 0, log-linear model, $\chi^2_1 = 6{\cdot}1$, $P = 0{\cdot}014$). The prevalence of diplostomula was also significantly higher in marine fish (Table 3, prevalence = 0·79) than in freshwater fish (prevalence = 0·49). Diplostomula were also more abundant in marine than freshwater fish (Table 4).

Parasites, antihelminthic treatment and growth

Of all parasites recovered the great majority (188/205) were diplostomula. In the rest of the analysis we therefore concentrate on the relationship between abundance of diplostomula, antihelminthic treatment and growth. Among the fish reared

Table 2. Results of a linear mixed model used to analyse the effect of rearing environment (fresh or salt) and antihelminthic treatment (treated or control) and their interaction on abundance of diplostomula in marine sticklebacks in experiment 1 (Scotland). The LMM had negative binomial errors and a logarithm link function. It included block (variance component = 0·61 ± 0·55), and enclosure nested within block (variance component = 0·29 ± 0·24) as random effects, in accordance with the split plot design of the experiment (see Methods). Means are back-transformed from GenStat parameter estimates; those for antihelminthic treatment and control are the means for fish reared in freshwater. Degrees of freedom were calculated in GenStat using the Kenward-Rogers algorithm

Explanatory variable	Level	Mean ± SE	Wald *F*	d.f.	*P*
Rearing environment	Fresh	3·8 ± 0·31	10·95	1, 43	0·002
	Salt	0 ± 0			
Antihelminthic treatment	Control	4·1 ± 0·91	4·46	1, 43	0·041
	Treatment	2·5 ± 0·89			
Environment × antihelminthic			0·18	1, 39	0·67

Table 3. Results of a log-linear model to analyse the effect of provenance (freshwater or marine) and antihelminthic treatment (treated or control), and their interaction on prevalence ('infected?', yes or no) of diplostomula in sticklebacks reared in enclosures in Loch na Buaile (freshwater) in experiment 1 (Scotland)

Explanatory variable	χ^2_1	*P*
Provenance × antihelminthic × infected?	0·04	0·84
Provenance × antihelminthic	0·19	0·66
Antihelminthic × infected?	0·05	0·82
Provenance × infected?	7·94	0·005

Table 4. Results of a linear mixed model used to analyse the effect of provenance (freshwater or marine) and antihelminthic treatment (treated or control), and their interaction on abundance of diplostomula in experiment 1 (Scotland). The LMM had negative binomial errors and a logarithm link function. It included block (variance component = 0·61 ± 0·47), and enclosure nested within block (variance component = 0·19 ± 0·15) as random effects, in accordance with the split plot design of the experiment (see Methods). Means are back-transformed from GenStat parameter estimates. Antihelminthic treatment and control means are for fish of freshwater provenance. For means in marine fish see Table 2. Degrees of freedom were calculated in GenStat using the Kenward-Rogers algorithm

Explanatory variable	Level	Mean	Wald *F*	d.f.	*P*
Provenance	Marine	3·8 ± 0·31	21·35	1, 18	< 0·001
	Freshwater	1·1 ± 0·16			
Antihelminthic treatment	Control	1·1 ± 0·28	4·09	1, 71	0·047
	Treatment	0·9 ± 0·40			
Provenance × antihelminthic			0·92	1, 67	0·34

Table 5. Results of a linear mixed model for growth in freshwater in experiment 1 (Scotland). Provenance, antihelminthic treatment, ln(diplostomula + 0·1) and their first order interactions were fitted as fixed effects. Block, and enclosure nested within block were fitted as random effects. A normal error function and identity link were used. Degrees of freedom were calculated in GenStat using the Kenward-Rogers algorithm

Explanatory variable	Wald *F*	d.f.	*P*
Provenance	0·07	1, 19	0·79
Antihelminthic treatment	4·29	1, 68	0·04
ln(diplostomula + 0·1)	1·28	1, 54	0·26
Provenance × ln(diplostomula + 0·1)	18·3	1, 70	< 0·001
Antihelminthic × ln(diplostomula + 0·1)	1·07	1, 68	0·30
Provenance × antihelminthic treatment	0·50	1, 70	0·48

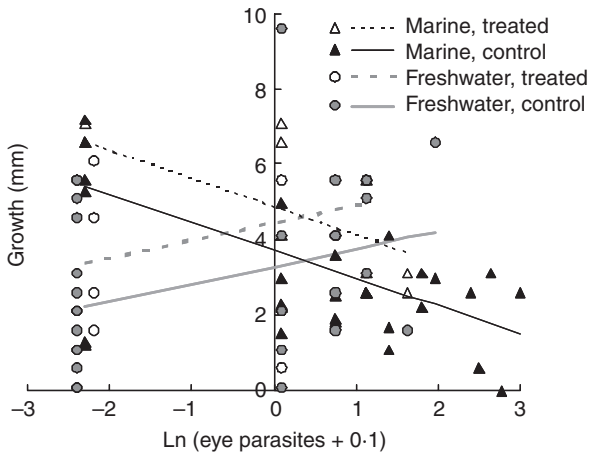

Fig. 2. Growth of sticklebacks reared in freshwater in experiment 1 (Scotland), in relation to the abundance of diplostomula [ln(diplostomula + 0·1)]. 'Growth' of each fish was calculated by subtracting from each individual's final length, the mean length of fish put into enclosures in that environment at the start of the experiment. Points for freshwater fish on the left hand side of the figure (burden of eye parasites = 0) have been slightly shifted in the figure to increase clarity.

in freshwater, there was no difference in prevalence of diplostomula between antihelminthic treated and control fish (treated = 0·64, control = 0·63, Table 3), but diplostomula were more abundant in control than in treated fish (Tables 2 and 4).

Although we cannot calculate actual individual growth rates properly, because fish were too small to be individually marked at the beginning of the experiment, they can be crudely estimated by subtracting the mean lengths of the samples measured at the beginning of the experiment (Table 1) from the length of each individual fish recovered at the end. 'Growth' of freshwater reared fish that had been treated with antihelminthic against parasites was greater than that of controls (Table 5, Fig. 2). Marine fish with more parasites in their eyes were smaller at the end of the experiment [slope of final length against ln(diplostomula + 0·1) = −0·74 ± 0·28, $t_{37} = -2{\cdot}64$, $P < 0{\cdot}025$], but freshwater fish with more parasites in the eyes were longer (slope = 0·46 ± 0·20, $t_{40} = 2{\cdot}29$, $P < 0{\cdot}05$).

For all fish of marine provenance, there was no difference in 'growth' between rearing environments (Wald $F_{1,47} = 0{\cdot}34$, $P = 0{\cdot}56$), antihelminthic treatments (Wald $F_{1,40} = 1{\cdot}77$, $P = 0{\cdot}19$) or for their interaction (Wald $F_{1,48} = 1{\cdot}73$, $P = 0{\cdot}19$, all in an LMM with normal errors and an identity link function. Block, and enclosure nested within block were included as random effects). However, when fresh-reared marines alone were considered, control fish that had not been treated against parasites grew less than antihelminthic treated fish (Fig. 3, LMM as above, but with antihelminthic treatment as the only fixed effect, Wald $F_{1,36} = 4{\cdot}57$, $P = 0{\cdot}04$).

EXPERIMENT 2, CANADA

At the end of the experiment 327 (157 marine fish) of the 400 original fish were recovered from the enclosures. The parasite supplementation treatment was partly successful in the sense that some fish were infected with *D. scudderi*. However, although mean abundance of *D. scudderi* tended to be higher in the parasite treatment enclosures (1·65 ± 1·05) than in the control enclosures (0·09 ± 0·03) this difference was not

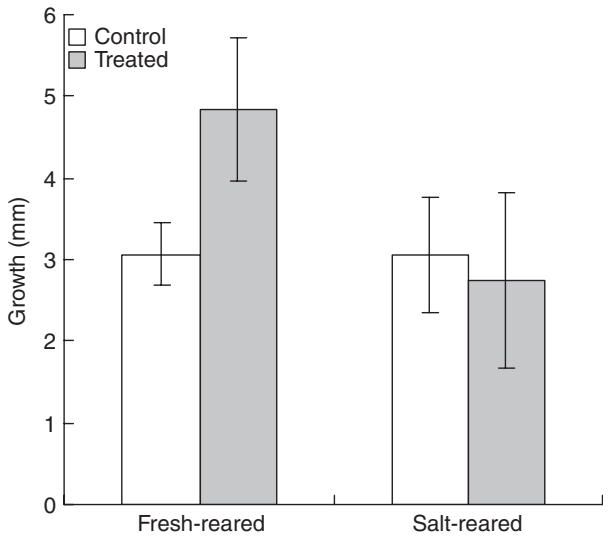

Fig. 3. Mean growth (±SE) of marine provenance fish in experiment 1 (Scotland), according to rearing environment and antihelminthic treatment. 'Growth' of each fish was calculated by subtracting from each individual's final length, the mean length of fish put into enclosures in that environment at the start of the experiment.

significant (Wald $F_{1,18} = 1{\cdot}1$, $P = 0{\cdot}31$). The interaction between population and treatment was not significant either (Wald $F_{1,307} = 0{\cdot}0$, $P = 0{\cdot}95$). The low level of infection in control enclosures probably resulted from some of the 'uninfected' *Stagnicola* that were added to these enclosures having pre-patent infections that developed during the course of the experiment, but might have originated from cercarial drift between enclosures or from snails native to the pond. The generally low abundance (see below) and lack of a clear difference in infection between supplementation and control enclosures means that it is difficult to make inferences about the effect of the parasite on growth. However, since the provenance 'treatment' was balanced at the level of enclosure, the experiment can still tell us about the relative susceptibility of marine and freshwater fish.

Diplostomum scudderi abundance in marine fish varied between 0 and 30 per fish, but were generally very low (mean of enclosure means = 0·9 ± 0·74 SE). Prevalence of *D. scudderi* in marine fish varied from 0 to 1 between enclosures (mean of enclosure means = 0·13 ± 0·05). Not a single freshwater fish ($n = 170$) was infected with *D. scudderi*. The difference in burdens between stickleback populations was very significant (Wald $F_{1,307} = 411{\cdot}6$, $P < 0{\cdot}001$).

Mean growth of freshwater fish was greater than that of marine fish (Wald $F_{1,15} = 35{\cdot}1$, $P < 0{\cdot}001$), but was unrelated to the prevalence of *D. scudderi* in the enclosures (Wald $F_{1,15} = 0{\cdot}6$, $P = 0{\cdot}45$) for either population (prevalence × provenance interaction, Wald $F_{1,15} = 0{\cdot}0$, $P = 0{\cdot}96$, LMM with mean 'initial length' of fish in each enclosure × provenance combination as a covariate). The growth of individual marine fish that were infected was unrelated to their burden of *D. scudderi* (Wald $F_{1,18} = 1{\cdot}1$, $P = 0{\cdot}31$, in an LMM with enclosure as a random effect, normal errors and an identity link function).

There was no difference in survival between the populations (Wald $F_{1,15} = 2{\cdot}5$, $P = 0{\cdot}13$), nor was survival related to the prevalence of *D. scudderi* in the enclosures (Wald $F_{1,15} = 0{\cdot}8$, $P = 0{\cdot}39$) for either population (prevalence × provenance interaction, Wald $F_{1,15} = 0{\cdot}7$, $P = 0{\cdot}42$, LMM with binomial errors and logit link function).

Discussion

Both experiments clearly show that marine fish which 'migrate' to freshwater are more susceptible to some freshwater parasites than are native freshwater fish. In experiment 1 parasite infection was associated with reduced growth. Since this reduction in growth is alleviated by treatment with antihelminthics, it is probably a direct consequence of infection. Size is strongly related to reproductive success in fish (Wootton 1979) and thus parasites probably reduce the fitness of marine fish when they disperse to freshwater. Therefore the results suggest that parasites could contribute to 'selection against migrants' when individuals from an ancestral, source population disperse to an environment with an established population of colonists. Although we believe that this is the first study to test the idea in an evolutionarily relevant context, there are now good reasons for thinking that parasites, and natural enemies in general, could represent an important source of selection against migrants, given that host local adaptation may be more common than once thought (Hoeksema & Forde 2008), and that the literature on biological invasions is beginning to recognize that biotic resistance may be at least as important as natural enemy release (Parker, Burkepile & Hay 2006). The results of this study certainly do not support the hypothesis of natural enemy release.

In experiment 1, marine sticklebacks became infected with significantly different parasites in the novel, freshwater, environment than in the ancestral, marine environment. The parasites acquired in freshwater were trematodes and cestodes, which were rare or absent in the marine environment, and may require quite different resistance mechanisms than the intracellular *G. anomala* that was the commonest parasite in saltwater. This result shows that parasite communities differ between environments, even when host genotypes are the same (or at least very similar). Experimental investigations of geographic variation in animal host-parasite interactions have normally concentrated on a single host and parasite species (although see Wegner, Reusch & Kalbe 2003). Such studies have frequently demonstrated that there can be substantial variation in host–parasite interactions among populations (Hoeksema & Forde 2008). Experimental investigations of the impact of natural enemies encountered by plants in different locations have commonly taken into account the whole community of herbivores or pathogens (Agrawal *et al.* 2005; Parker, Burkepile & Hay 2006). This is rare among studies of the parasites of animals although the substantial variation in parasite communities among host populations, documented by observation (Poulin 2007) suggests that hosts which disperse to novel environments may often encounter diverse new parasite species. The

consequences of this for the evolution of resistance in different host populations is an open question which has received little attention (but see Lindstrom *et al*. 2004; Kalbe & Kurtz 2006; Bryan-Walker, Leung & Poulin 2007; Scharsack *et al*. 2007). Local adaptation of immunity or parasite resistance may have important consequences for the evolution of reproductive isolation, for example by resulting in the reduced fitness of hybrids (Moulia 1999; Bomblies *et al*. 2007).

In both experiments, freshwater sticklebacks reared in freshwater became infected with significantly fewer parasites than marine sticklebacks reared in the same environment. This result shows that host populations can have different susceptibility to the same parasites encountered within the same environmental conditions. The fact that the derived, freshwater fish were less susceptible to freshwater parasites than marine fish suggests that the freshwater fish may have evolved reduced susceptibility to these parasites, although the mechanism of 'resistance' is not clear. It could be immunological resistance, but could also result from changes in behaviour, or avoidance of certain microhabitats or food items. Local adaptation of hosts to their natural enemies may have important consequences for the long term dynamics of colonizing populations. This has tended to be overlooked in the ecological literature on invasions (although see Hawkes 2007), something which we consider further below.

Alternatively, the increased susceptibility of marine fish might be the result of chronic stress brought about by being raised in freshwater (Marchinko & Schluter 2007). To the extent that the change in salinity is one that necessarily accompanies the invasion of freshwater, this would not alter our conclusion that parasites contribute to selection against migrants in this system. In any case, the marine fish used in this experiment came from an anadromous population that naturally experiences low salinity (at least as low as 5 ‰ salt, A. D. C. MacColl, unpublished data) as both juveniles and breeding adults, so it is likely that their physiologies deal well with changing salinity. This is supported by the fact that, as best as we can estimate, growth rates of marine fish in freshwater were similar to those in saltwater, when not treated with antihelminthics, but higher than those in saltwater when parasite burdens were reduced by antihelminthic treatment. As an aside, it is interesting that the antihelminthic treatment had a significant effect on the abundance of diplostomules, which live in the retina and humour of the eye. Accepted wisdom in parasitology suggests that the eye is an immunologically privileged site, where parasites can escape from such treatment or from the host immune system. The results presented here suggest either that diplostomules are susceptible to antihelminthic while in the stickleback eye, or that parasites were only killed during the course of their migration there from wherever they penetrated the skin.

In experiment 1, marine fish reared in freshwater grew to smaller size when infected with more parasites. The fact that antihelminthic treated fish experienced increased growth relative to control fish suggests that parasites were the cause of this reduced growth. In contrast, freshwater fish with more parasites grew to larger size. Increased growth of infected hosts is a common feature of the interaction between molluscs and their trematode parasites (Minchella 1985; Ward, Goater & Mikos 1997; Miura *et al*. 2006), including *Diplostomum* (Ballabeni 1995). Enhanced growth in response to infection has also been recorded in fish hosts, including sticklebacks (Arnott, Barber & Huntingford 2000; Loot *et al*. 2002), although this seems to depend on food availability (Barber *et al*. 2008). We have also documented increased growth of sticklebacks in the lab, following artificial infections with *Diplostomum spathaceum* (J. De Roij and A.D.C. MacColl, unpublished data). The response may be (i) a non-adaptive side effect of infection or (ii) it may constitute a form of manipulation by the parasite, or (iii) of resistance or adaptive life-history alteration by the host (Taskinen 1998). The latter two hypotheses predict that such responses will be more common in sympatric host–parasite interactions, as we have found here. A similar pattern was documented by Ballabeni (1995) in the interaction between the freshwater snail *Lymnaea peregra* and the parasite *Diplostomum phoxini*.

It had become an accepted wisdom in the field of invasion ecology that escape from all kinds of natural enemies, including parasites, facilitates colonization of novel environments by host organisms ('natural enemy release', Lafferty *et al*. 2005). Torchin *et al*. (2003) and Mitchell & Power (2003) showed that populations of animals and plants, respectively, established beyond their natural distributions through human actions, suffer less from parasitism than do the same species within their native distribution. However, recent meta-analyses have cast doubt on the generality of natural enemy release (Maron & Vila 2001; Colautti *et al*. 2004; Parker, Burkepile & Hay 2006). This study lends further supports that to the idea that biotic resistance due to natural enemies may be as common, or more so, than enemy release.

There are good *a priori* reasons to doubt the generality of natural enemy release and to treat observational studies of it with caution: (i) They are biased, in the sense that they only include data from successful 'colonizations' and can tell us nothing about the role played by parasites in the undoubtedly large number of failed colonizations. (ii) They consider only the short term change in invaders' natural enemy communities. In this sense, natural enemy release is an ecological hypothesis which ignores the longer term evolutionary dynamics of the interaction between natural enemies and colonizers in novel environments. Even species that experience release at first, may accrue novel enemies with the passage of time (Hawkes 2007), and this may lead to the collapse of these colonizing populations or the evolution of novel forms of resistance (Ricklefs 2005; Siemann, Rogers & Dewalt 2006). (iii) There is a further reason to suspect that the role of natural enemies may be different in natural colonizations than in anthropogenic invasions. Natural colonizations are likely to be over shorter distances than the trans-continental and trans-oceanic scales of anthropogenic invasions. As a result natural colonizations may be more likely to remain linked to their ancestral range by the continued migration of conspecifics, and may also be more likely to end up in geographic proximity with very closely related taxa. This could

well mean that they are less likely to lose the enemies from their native range and more likely to acquire novel ones in the new range. Continuing gene flow between a colonizing host population and its native range is also predicted by theory to make local adaptation to parasites more likely in the hosts (Hoeksema & Forde 2008). This gives rise to the interesting and counter-intuitive possibility that gene flow in the early stages of population separation of hosts could favour the restriction of long-term gene flow, by allowing local adaptation of hosts and reducing the relative fitness of migrants.

The results reported here have implications for our understanding of the mechanisms that drive divergent selection during adaptive radiation and speciation. Investigation of the ecological mechanisms responsible for such divergence has concentrated on the role of resource exploitation, competition and the associated evolution of trophic traits (Schluter 2000). The role played by natural enemies is much less well known, and again, parasites have been ignored as agents of selection (Vamosi 2005; although see Scharsack *et al*. 2007).

This is surprising given the ubiquity of parasites in natural systems and their acknowledged effects on fitness, but reflects a wider lack of study of the role of parasites in community functioning and as selective agents (Thompson, Mouritsen & Poulin 2005; Kuris *et al*. 2008). The many, dramatic examples of parasites invading a novel environment and devastating local host populations strongly suggest that dispersal to the parasites original distribution by the host organism would have been impossible. It is unlikely that most Hawaiian birds could have colonized continental areas because of avian malaria and other diseases, or that African ungulates could have invaded India because of rinderpest (Spinage 2003). We have shown here that parasites can cause selection against migrants that could contribute to reducing gene flow between populations and hence to the accumulation of reproductive isolation (Hendry 2004; Nosil, Vines & Funk 2005).

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References

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology*, **86**, 2979–2989.

Arnott, S.A., Barber, I. & Huntingford, F.A. (2000) Parasite-associated growth enhancement in a fish-cestode system. *Proceedings of the Royal Society B-Biological Sciences*, **267**, 657–663.

Balkau, B.J. & Feldman, M.W. (1973) Selection for migration modification. *Genetics*, **74**, 171–174.

Ballabeni, P. (1995) Parasite-induced gigantism in a snail: a host adaptation? *Functional Ecology*, **9**, 887–893.

Barber, I. (2007) Host-parasite interactions of the three-spined stickleback. *Biology of the Three-Spined Stickleback* (eds S. Östlund-Nilsson, I. Mayer & F.A. Huntingford), pp. 271–317. CRC Press, Boca Raton.

Barber, I., Wright, H.A., Arnott, S.A. & Wootton, R.J. (2008) Growth and energetics in the stickleback-*Schistocephalus* host-parasite system: a review of experimental infection studies. *Behaviour*, **145**, 647–668.

Bell, M.A. & Foster, S.A. (1994) Introduction. *The Evolutionary Biology of the Threespine Stickleback* (eds M.A. Bell & S.A. Foster), pp. 1–27. OUP, New York.

Bomblies, K., Lempe, J., Epple, P., Warthmann, N., Lanz, C., Dangl, J.L. & Weigel, D. (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLoS Biology*, **5**, 1962–1972.

Borer, E.T., Hosseini, P.R., Seabloom, E.W. & Dobson, A.P. (2007) Pathogen-induced reversal of native dominance in a grassland community. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 5473–5478.

Bryan-Walker, K., Leung, T.L.F. & Poulin, R. (2007) Local adaptation of immunity against a trematode parasite in marine amphipod populations. *Marine Biology*, **152**, 687–695.

Bykhovskaya-Pavlovskaya, I.E., Gusev, A.V., Dubinina, M.N., Izyumova, N.A., Smirnova, T.S., Sokolovskaya, I.L., Shtein, G.A., Shul'man, S.S. & Epshtein, V.M. (1964) *Key to Parasites of Freshwater Fish of the U.S.S.R.* Israel Program for Scientific Translations, Jerusalem.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & Macisaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721–733.

Coyne, J.A. & Orr, H.A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA.

Darwin, C. (1859) *The Origin of Species by Means of Natural Selection*. John Murray, London.

Dickinson, H. & Antonovics, J. (1973) Theoretical considerations of sympatric divergence. *American Naturalist*, **107**, 256–274.

Dobzhansky, T. (1951) *Genetics and the Origin of Species*. Columbia University Press, New York.

Engelkes, T., Morrien, E., Verhoeven, K.J.F., Bezemer, T.M., Biere, A., Harvey, J.A., Mcintyre, L.M., Tamis, W.L.M. & Van Der Putten, W.H. (2008) Successful range-expanding plants experience less above-ground and below-ground enemy impact. *Nature*, **456**, 946–948.

Galwey, N.W. (2006) *Introduction to Mixed Modelling: Beyond Regression and Analysis of Variance*. Wiley, Chichester.

Gulland, F.M.D., Albon, S.D., Pemberton, J.M., Moorcroft, P.R. & Clutton-Brock, T.H. (1993) Parasite associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society B-Biological Sciences*, **254**, 7–13.

Hawkes, C.V. (2007) Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. *American Naturalist*, **170**, 832–843.

Hendry, A.P. (2004) Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research*, **6**, 1219–1236.

Hoeksema, J.D. & Forde, S.E. (2008) A meta-analysis of factors affecting local adaptation between interacting species. *American Naturalist*, **171**, 275–290.

Kalbe, M. & Kurtz, J. (2006) Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke *Diplostomum pseudospathaceum*. *Parasitology*, **132**, 105–116.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164–170.

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E. & Lafferty, K.D. (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, **454**, 515–518.

Lafferty, K.D., Smith, K.F., Torchin, M.E., Dobson, A.P. & Kuris, A.M. (2005) The role of infectious diseases in natural communities: what introduced species tell us. *Species Invasions. Insights into Ecology, Evolution, and Biogeography* (eds D.F. Sax, J.J. Stachowicz & S.D. Gaines), pp. 111–134. Sinauer Associates, Sunderland, MA.

Lindstrom, K.M., Foufopoulos, J., Parn, H. & Wikelski, M. (2004) Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proceedings of the Royal Society B-Biological Sciences*, **271**, 1513–1519.

Loot, G., Poulin, R., Lek, S. & Guegan, J.F. (2002) The differential effects of *Ligula intestinalis* (L.) plerocercoids on host growth in three natural populations of roach, *Rutilus rutilus* (L.). *Ecology of Freshwater Fish*, **11**, 168–177.

MacColl, A.D.C. (2009) Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, **32**, 153–160.

Makinen, H.S., Cano, J.M. & Merila, J. (2006) Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Molecular Ecology*, **15**, 1519–1534.

Marchinko, K.B. & Schluter, D. (2007) Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution*, **61**, 1084–1090.

Maron, J.L. & Vila, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361–373.

Mayr, E. (1942) *Systematics and the Origin of Species*. Columbia University Press, New York.

McKinnon, J.S. & Rundle, H.D. (2002) Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution*, **17**, 480–488.

Minchella, D.J. (1985) Host life-history variation in response to parasitism. *Parasitology*, **90**, 205–216.

Mitchell, C.E. & Power, A.G. (2003) Release of invasive plants from fungal and viral pathogens. *Nature*, **421**, 625–627.

Miura, O., Kuris, A.M., Torchin, M.E., Hechinger, R.F. & Chiba, S. (2006) Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 1323–1328.

Moulia, C. (1999) Parasitism of plant and animal hybrids: are facts and fates the same? *Ecology*, **80**, 392–406.

Nosil, P. & Crespi, B.J. (2006) Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 9090–9095.

Nosil, P., Vines, T.H. & Funk, D.J. (2005) Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**, 705–719.

Parker, J.D., Burkepile, D.E. & Hay, M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, **311**, 1459–1461.

Parker, I.M. & Gilbert, G.S. (2007) When there is no escape: the effects of natural enemies on native, invasive, and noninvasive plants. *Ecology*, **88**, 1210–1224.

Poulin, R. (2007) *Evolutionary Ecology of Parasites*. Princeton University Press, Princeton.

Ricklefs, R.E. (2005) Taxon cycles: insights from invasive species. *Species Invasions. Insights into Ecology, Evolution, and Biogeography* (eds D.F. Sax, J.J. Stachowicz & S.D. Gaines), pp. 165–199. Sinauer Associates, Sunderland, MA.

Scharsack, J.P., Kalbe, M., Harrod, C. & Rauch, G. (2007) Habitat-specific adaptation of immune responses of stickleback (*Gasterosteus aculeatus*) lake and river ecotypes. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1523–1532.

Schluter, D. (1994) Experimental evidence that competition promotes divergence in adaptive radiation. *Science*, **266**, 798–801.

Schluter, D. (2000) *The Ecology of Adaptive Radiation*. OUP, Oxford.

Siemann, E., Rogers, W.E. & Dewalt, S.J. (2006) Rapid adaptation of insect herbivores to an invasive plant. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 2763–2769.

Spinage, C.A. (2003) *Cattle Plague: A History*. Kluwer Academic/Plenum, New York.

Taskinen, J. (1998) Influence of trematode parasitism on the growth of a bivalve host in the field. *International Journal for Parasitology*, **28**, 599–602.

Thompson, R.M., Mouritsen, K.N. & Poulin, R. (2005) Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *Journal of Animal Ecology*, **74**, 77–85.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., Mckenzie, V.J. & Kuris, A.M. (2003) Introduced species and their missing parasites. *Nature*, **421**, 628–630.

Vamosi, S.M. (2005) On the role of enemies in divergence and diversification of prey: a review and synthesis. *Canadian Journal of Zoology*, **83**, 894–910.

Ward, P.I., Goater, C.P. & Mikos, M. (1997) Shell variation in sympatric freshwater *Lymnaea peregra* and *L. ovata* (Gastropoda: Lymnaeidae). *Biological Journal of the Linnean Society*, **61**, 139–149.

Wegner, K.M., Reusch, T.B.H. & Kalbe, M. (2003) Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of Evolutionary Biology*, **16**, 224–232.

Wootton, R.J. (1979) Energy costs of egg production and environmental determinants of fecundity in teleost fishes. *Symposia of the Zoological Society of London*, **44**, 133–159.

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