Parasites contribute to ecologically dependent postmating isolation in the adaptive radiation of three-spined stickleback

Aliya El Nagar† and Andrew D. C. MacColl

School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

ADCM, 0000-0003-2102-6130

Spatial variation in parasitic infections is common, and has the potential to drive population divergence and the reproductive isolation of hosts. However, despite support from theory and model laboratory systems, little strong evidence has been forthcoming from the wild. Here, we show that parasites are likely to cause reproductive isolation in the adaptive radiation of three-spined stickleback. Adjacent wild populations on the Scottish island of North Uist differ greatly and consistently in the occurrence of different parasites that have substantial effects on fitness. Laboratory-reared fish are more resistant to experimental infection by parasite species from their own population. Furthermore, hybrid backcrosses between the host populations are more resistant to parasites from the parental population to which they are more closely related. These patterns provide strong evidence that parasites can cause ecological speciation, by contributing to selection against migrants and ecologically dependent postmating isolation.

1. Background

The ecological model of speciation, which proposes that reproductive isolation between populations occurs as a result of divergent adaptation to different environments, is widely accepted as a major explanation for how new species arise [1,2]. However, many questions still exist about how it works and the extent of its importance in comparison with other mechanisms [2,3]. One important question concerns which aspects of ecology drive the divergence between populations that initiates the process. Most effort to date has concentrated on competition and the acquisition of food, predation, and the role of the environment in mate choice [4–7]. The dynamic nature of the relationship between hosts and parasites [8], and its potential to drive divergence, suggests a role for parasites in host speciation [9–11]. While there is both theoretical underpinning for this idea [12,13] and support from model laboratory systems [14], little conclusive evidence has been forthcoming from natural populations [15]. Three prerequisites are necessary for parasites to contribute to speciation: that parasitism should differ within or between host populations, that these differences should remain consistent, and that parasites should impose fitness costs on the host [15]. These conditions are not sufficient in themselves to show that parasites do contribute to host speciation; in addition, it is necessary to document specific reproductive isolating mechanisms.

Two forms of reproductive isolation are unique predictions of the ecological speciation model [2]. Firstly, selection against immigrants, a form of premating isolation, occurs when immigrants to a population experience reduced fitness because they are not adapted to local environmental conditions [16,17]. Secondly, ecologically dependent postmating isolation occurs when hybrids between divergent populations are inferior for purely ecological reasons. Postmating isolation happens when individuals from different populations...
mate, but the offspring they produce are inferior in some way. This inferiority may arise because of intrinsic genetic incompatibilities between populations, or because the hybrid individuals experience an ecologically dependent mismatch to the parental environments, as a result of divergent local adaptation. Identification of these two types of postmating isolation is an important task for speciation biologists because the former can arise during several processes that lead to speciation, while the latter is exclusively a prediction of the ecological speciation model [2,18]. Separation of the two is not trivial, because reduced fitness of certain types of hybrids (notably F1s) is an expectation of both models. However, the two can be reliably separated by quantifying the performance of reciprocal backcrosses (electronic supplementary material, figure S1) in both parental environments [19]. As the different backcross types share the same degree of hybridity, the intrinsic hypothesis predicts no difference in their performance. By contrast, the ecological hypothesis predicts that backcrosses will perform best in the environments of the parental types to which they are more closely related, resulting in a switch in relative performance between environments [19]. Statistically, this switch reveals itself as an interaction between backcross type and environment when metrics of performance in the different environments are analysed [20].

Although powerful, the reciprocal backcross method has seldom been used as a research technique [20,21], perhaps because of the effort required or the difficulty of raising backcrosses between divergent populations [2]. Extension of the reciprocal backcross method to include parental types, F1s, and F2s, allows the use of line-cross analysis to explore the quantitative genetic basis of variation in (resistance) phenotypes [22].

The adaptive radiation of three-spined stickleback (Gasterosteus aculeatus) in waterbodies on the Scottish island of North Uist provides an ideal opportunity for testing the role of parasites in population divergence and speciation, given the variation in parasites between populations [23] and the occurrence of closely related, but reproductively isolated, sympatric ecotypes of stickleback (see below).

In order to infer a sufficient role of parasites in host speciation, we first show that the three necessary prerequisites for parasites to contribute to speciation are true for an adjacent pair of stickleback populations on North Uist, which we call CHRU (from a small freshwater loch) and OBSM (from a brackish lagoon) (figure 1). CHRU and OBSM exhibit substantial genetic divergence (see Material and methods). A third population (`OBSE`) which is sympatric with, but reproductively isolated from, OBSM is morphologically similar to CHRU, demonstrating the potential for speciation in North Uist stickleback (figure 1).

We then apply the reciprocal backcross method for the first time to the question of whether parasites contribute to ecologically dependent postmating isolation, by exposing backcrosses between CHRU and OBSM to artificial infection by both a CHRU-specific parasite and an OBSM-specific parasite. We also compare the resistance of the parental types in order to add to existing work [24] suggesting that parasites could cause selection against immigrants in our system. These tests allow strong inference about the role of parasites in ecological speciation, because they arise from predictions that are unique to the model [19–21]. We show that parental types are much more resistant to the parasite that infects their own population. The resistance of backcrosses switches, depending on the parasite to which they are exposed, such that they are most resistant to the parasites from the more closely related parental population.

2. Material and methods

(a) Study area

On the island of North Uist, Scotland, many isolated freshwater loch (lake) populations of stickleback show a high degree of phenotypic variation [25,26], which has probably evolved since the end of the last glaciation, approximately 16 000 years ago, before which North Uist was covered in ice [27]. Marine three-spined stickleback, from which the freshwater fish are probably derived, visit coastal brackish lagoons and some low-lying
freshwater habitats to breed, but do not generally hybridize with the resident fish, which inhabit these waterbodies year round. We focus on the potential for parasites to contribute to reproductive isolation between an adjacent pair of waterbodies (figure 1), Loch a Chadha Ruaidh (CHRU, 57°36′ N; 7°12′ W), which supports a freshwater population of stickleback, and Ob nan Stearnain (OBSM, 57°36′ N; 7°10′ W), a brackish lagoon 1.5 km distant. CHRU, at an altitude of about 15 m and connected by a stream to the sea less than 400 m away, is not presently visited by marine stickleback, although the extant population was probably established from the sea, and migratory fish species (catadromous European eels, Anguilla anguilla) are occasionally present in the loch, so the potential for secondary contact is high. OBSM contains one of the nearest breeding populations of marine stickleback, and also supports a population of resident stickleback with freshwater morphology. We refer to the latter as saltwater residents, and give them the code OBSE. Given their phenotype (figure 1), it seems likely that this population underwent a period of evolution in freshwater before re-encountering salt water, either as a consequence of dispersal or because of rising sea levels. The sympatry of OBSE and OBSM during the breeding season, with little or no hybridization (approx. 5% F1), is important in the present context, because it demonstrates that there is reproductive isolation between fish with marine and freshwater morphology following secondary contact. Values of population genetic differentiation (Fr calculated from eight putatively neutral microsatellite markers spread across the genome) suggest that CHRU fish are substantially diverged from both OBSE (0.38, p < 0.0001) and OBSM (0.37, p < 0.0001), which are much less, but still significantly, differentiated from each other (0.03, p < 0.0001) [28]. The apparent contradiction here, between our claim of strong reproductive isolation between OBSM and OBSE and the low Fr value arises easily when effective population sizes are large (which they are likely to be for the many interconnected populations of stickleback living in saltwater), because standard population genetics tells us that Fr is determined by the absolute number of migrants exchanged per generation [29], while reproductive isolation is defined by the proportion of hybrids [18].

(b) Field methods

Wild fish were caught in unbaited minnow traps (Gee’s, Dynamic Aqua, Vancouver) set for 24 h around the margins of waterbodies in water approximately 0.3–3 m deep. Captured fish were emptied into buckets and a haphazardly selected sample returned to the laboratory in styrofoam boxes. Fish were assayed for parasite infections within 24 h (usually within 8 h). They were first euthanized by an overdose of MS222, weighed and measured. External surfaces were carefully examined under a 10×–40× dissecting microscope, and all Gyrodactylus and other ectoparasites (including Cryptocotyle sp.) counted. Eyes were removed and dissected to count Diplostomum sp. and Apatemon gracilis. Schistoscephalus solidus in the body cavity and Proteocephalus filicollis in the intestine were also recorded. All fish had their liver removed, blotted and the wet weight recorded. Hepatosomatic index (HSI), a measure of medium-term energy reserves [30], was calculated as (liver weight × 100/fish weight) (Schistoscephalus weight + liver weight).

(c) Crossing methods

Fish that were used in artificial infection experiments were raised in the laboratory from wild (grand)parents, according to standard in vitro methods. Briefly, testes were removed from mature wild males, minced and, in 1 ppt marine salt solution, mixed with eggs stripped from fully gravid wild females from both their own and the other population, to create pure parental and F1 crosses (electronic supplementary material, figure S1). Fertilized eggs were returned to the aquarium at the University of Nottingham, where they were reared to maturity (approx. 12–14 months). Eggs and sperm from these mature fish were then used to produce a full range of cross types (pure parents, F1, F2, and reciprocal backcrosses) between the three populations, without inbreeding.

(d) Experimental infections

Laboratory-raised fish, representing the full range of cross types, were exposed to infection by Gyrodactylus sp. and Diplostomum sp. in two experiments. In our experimental infections, we used parasites that were allopatric to the ones to which the fish are naturally exposed. This removes any possibility that our results are simply due to host–parasite local adaptation, making our conclusions more generic. For Diplostomum infections, pond snails (Lymnaea peregra) were collected from a lake on the campus of the University of Nottingham, returned to the laboratory and exposed to bright light in a change of water, which elicits the release of Diplostomum cercariae. Each fish was exposed to a dose of 20 cercariae in 11 l of aquarium water. Fish were euthanized after 48 h, their eyes removed and the total number of cercariae present was counted. For Gyrodactylus infections, fish were individually housed in 10 l tanks. Each fish was anaesthetized and infected with two Gyrodactylus worms removed from wild fish caught at Barkby Brook, Syston, Leicestershire, UK (52°42′ N; 1°04′ W). Gyrodactylus were then counted every fourth day until the infection was lost (which takes up to six weeks). Peak infection was used as the metric of susceptibility, although the use of other metrics of infection severity, such as number of worms at day 28 or area under the infection curve (the sum of all counts for a fish for the whole infection period), makes no difference to the outcome of analyses. The CHRU × OBSM Gyrodactylus experiment used 58 fish from 26 families. The CHRU × OBSM Diplostomum experiment used 68 fish from 30 families.

Under UK Government Home Office regulations, which determine the treatment of animals in research, it is illegal to subject vertebrates to treatments where death is an endpoint: animals must be euthanized before this, e.g. if they begin to show symptoms which suggest the onset of severe infections. Gyrodactylus infections of stickleback sometimes result in secondary infection by bacteria, fungi, and protozoa. These infections develop rapidly and are commonly fatal in the absence of intervention. In our experiments, we immediately intervened in such cases by euthanizing fish, as legally required. However, we assumed that these fish would have died in the absence of intervention, and we used such cases of euthanasia to calculate ‘case fatality rates’ (CFR).

(e) Statistical analysis

Parasite occurrence data from wild fish were analysed in GenStat 15 using generalized linear models (GLMs) with appropriate error distributions and link functions. Initially, we analysed data from artificial infection experiments using generalized linear mixed models (GLMMs) that included ‘family’ as a random term, but family never accounted for a significant proportion of the variance, and we reverted to the use of GLMs. In line-cross analyses, we followed Lynch & Walsh [22]. The Rundle–Whitlock method [19] for testing for ecologically dependent postmating isolation depends on showing that there is an interaction between backcross type and environment in determining performance [20]. Carrying out such an analysis requires the measurement of performance on the same scale in different environments. Our measures of performance are susceptibility to infection, but to two different parasites, and therefore on different scales. We therefore converted them into standardized deviates
(z scores) [31], which can be understood as ‘environment specific susceptibility to infection’.

3. Results

Two different parasites, *Gyrodactylus* sp. (probably *arcuatus*) and *Diplostomum* sp. (probably *gasterostei*) (ectoparasitic and ocular trematodes, respectively) differed strongly in prevalence between CHRU and OBSM (figure 2a,b and table 1): *Gyrodactylus* was very common on fish in brackish water, but almost absent from the freshwater loch. By contrast, *Diplostomum* infected the majority of fish in freshwater, but was completely absent in brackish water. These patterns have remained consistent over several years, in fulfilment of the second necessary prerequisite. These differences are to some extent constrained to be true because both parasites are sensitive to changes in salinity. *Diplostomum* is not transmitted in saltwater because the freshwater snails (*Radix* spp.) that are its intermediate host are not present. *Gyrodactylus* does exist in freshwater, but is generally less prevalent and abundant than in salt (ADC MacColl 2007–2014, unpublished data). Both parasites are individual elements of larger parasite communities which differed dramatically in mean abundance.
in our study, intake, leading to a reduced metabolic rate and enlarged liver studies have shown that infections can interfere with food cause selection against immigrants. Previous experimental crosses (figure 2f; for Gyrodactylus, Wald $F_{1,10} = 9.28$, $p = 0.012$; for Diplostomum, Wald $F_{1,20} = 58.1$, $p < 0.001$). Full quantitative genetic line-cross analysis of our data for all crosses (parentals, F1s, F2s, and backcrosses; electronic supplementary material, table S1) reveals the genetic architecture of differences between environments in host susceptibility. In Gyrodactylus infections, for both peak infection and CFR, differences are additive, whereas for Diplostomum there are also elements of dominant susceptibility. Such a pattern of dominance will also contribute to the inferiority of hybrids in the freshwater environment, further strengthening reproductive isolation.

4. Discussion

We have shown that parasites with known effects on fitness can differ greatly and consistently between adjacent populations. Host populations have higher, presumably evolved, resistance to the parasites in their own environment, but are susceptible to parasites that they seldom or never encounter. Resistance of backcrosses between the populations switches between environments, such that it is higher in the environment of the parental population to which each backcross type is more closely related. Fish would therefore be at a disadvantage if they migrated between populations, fuelling selection against immigrants. Crosses between the populations would also be at an disadvantage in the environments of either parental type, not just because they are hybrids per se, but because they are less well adapted than the parents to the ecological (parasitological) conditions existing in those environments. Thus, in the event of secondary contact between our populations, environment-specific parasites would be likely to contribute to reproductive isolation. Because neither Gyrodactylus sp. nor Diplostomum sp. is contracted through the diet, the adaptation to differences in infection is likely to be primary, and not a secondary consequence of changes in feeding ecology [15]. The total effect of environment-specific parasites on divergence and speciation in our system is likely to be greater than we have shown here, because there are at least another four parasites which are almost completely environment specific.

It has previously been shown that parasites of stickleback can have a negative effect on growth when they are translocated between locations, although previous experiments or effects have tended to be directional, rather than reciprocal [17,24]. Such effects, and those we have documented here, may be at least partly the result of differences in major histocompatibility (MHC) genes, which have been shown to be under divergent selection in stickleback [40].

In stickleback, the transition from salt- to freshwater environments is clearly associated with many ecological changes and with rapid adaptation and even speciation [41]. It is important in our study system, because both Gyrodactylus and Diplostomum are sensitive to salinity. Among the genetic changes that occur when stickleback adapt to freshwater, some of the clearest involve changes in genes that are likely

Table 1. Summary of statistical analyses of differences between CHRU and OBSM in prevalence and abundance of six common parasite species. Samples of at least 20 fish were taken from each population in 2011 and 2013. Population differences in prevalence were compared with Z tests; those for abundance, with GLMs with negative binomial errors and logarithm link functions.

| parasite          | statistic | d.f. | $p$-values |
|-------------------|-----------|------|------------|
| prevalence        | Z         | 1,94 | < 0.001    |
| Gyrodactylus sp.  | 9.34      | 1,94 | < 0.001    |
| Diplostomum sp.   | 8.03      | 1,94 | < 0.001    |
| abundance         | Wald F    |      |            |
| Gyrodactylus sp.  | 416.31    | 1,94 | < 0.001    |
| Diplostomum sp.   | 263.47    | 1,94 | < 0.001    |
| Apatemon gracilis | 130.50    | 1,94 | < 0.001    |
| Schistocephalus solidus | 44.30 | 1,94 | < 0.001    |
| Proteocephalus filicollis | 42.98 | 1,94 | < 0.001    |
| Cryptocotyle sp.  | 129.48    | 1,94 | < 0.001    |

between the two populations (electronic supplementary material, figure S2; table 1).

Gyrodactylus and Diplostomum have well documented effects on the fitness of fish hosts, largely through effects on survival, fulfilling the third necessary prerequisite. Gyrodactylus is one of the most widespread and common of all fish parasite genera. They have a direct life cycle with no need for alternative hosts, can reproduce parthenogenetically, and worms are born pregnant, giving the parasite a high potential for a rapid increase in numbers. Gyrodactylus salaris is well known for its disastrous effects on Norwegian salmon fisheries [32]. Previous studies of Gyrodactylus infections on a variety of fish species have shown that higher abundance of the parasite is associated with reduced survival of the host and other pathological effects [33–35]. In our experiments, parental CHRU and CHRU backcross fish exhibited higher CFRs following infection than other types of crosses (figure 2c; electronic supplementary material, table S1).

Diplostomum is also a widespread and common parasite of freshwater fish, with pathological effects on hosts that lead to reduced survival [36–38]. It has previously been shown that infections reduce growth rate in marine fish experimentally translocated to freshwater [24], suggesting that parasites cause selection against immigrants. Previous experimental studies have shown that infections can interfere with food intake, leading to a reduced metabolic rate and enlarged liver size [39]. In our study, Diplostomum infection in wild caught fish from CHRU was associated with liver enlargement (greater HSI, figure 2d, GLM, $F_{1,81} = 17.86$, $p < 0.0001$), suggesting metabolic consequences of infection [39]. Thus, it seems likely that both Gyrodactylus and Diplostomum reduce fitness, although this remains to be demonstrated in wild stickleback. Doing so would fulfill the necessary conditions for parasites to contribute to host speciation for CHRU and OBSM, but the results presented here are strongly suggestive.

The susceptibility of backcrosses to experimental infection clearly switched depending on the parasite to which they were exposed, with each backcross type (i.e. CHRU BC or OBSM BC) performing similarly to the most closely related parental type (figure 2e,f; GLM: environment $\times$ backcross type, $F_{1,30} = 19.27$, $p < 0.001$, backcross type $F_{1,31} = 0.39$, $p = 0.54$, environment $F_{1,31} = 0.15$, $p = 0.71$). This strongly suggests that hybrids between CHRU and OBSM, should they occur in the wild, would have reduced fitness in the environment of either parental type. We also found that the susceptibility to experimental infection by both Gyrodactylus and Diplostomum differed significantly between pure CHRU and OBSM, with fish being more susceptible to foreign parasites in both comparisons (figure 2f; for Gyrodactylus, Wald $F_{1,10} = 9.28$, $p = 0.012$; for Diplostomum, Wald $F_{1,20} = 58.1$, $p < 0.001$).
to have an effect in the immune system such as TNFfs13b (BAFF), GARP, and Muc5b [42,43]. These changes could have implications for parasite resistance although the functional significance of them is unknown.

Hybrids between different taxa (commonly congeneric species) have previously been shown to have increased susceptibility to infection [44] but reciprocal exposure of (within species) host populations to (different species of) parasites from different environments does not previously seem to have been carried out. This may result from the difficulty of assembling the information to satisfy the prerequisite criteria [15] and carrying out the necessary crossing and infection experiments. We hope that our work may encourage others to see this as worthwhile, and to explore whether the contribution of spatial variation in parasite infection to the evolution of reproductive isolation is a general one.

Ethics. All work was carried out with University of Nottingham ethical approval and under licence from the UK Government Home Office.

Data accessibility. All data have been deposited in Dryad, http://dx.doi.org/10.5061/dryad.hlq1s.

Authors’ contributions. A.E.N. conducted fieldwork, designed and carried out experiments, analysed data, and contributed to writing the manuscript. A.D.C.M. conceived the project, conducted fieldwork, designed and supervised experiments, analysed data, and wrote the manuscript.

Competing interests. We have no competing interests.

Funding. The work was supported by the UK Natural Environment Research Council (NE/CI7525/1) and the University of Nottingham.

Acknowledgements. We are grateful to North Uist Estates for access to lochs. Becca Young, Job de Roij, and Daniele D’Agostino gave assistance with fieldwork, and the manuscript was improved by comments from Isabel Magalhaes, Sara Goodacre, Andrew Hendry, and his laboratory and two anonymous reviewers.

References

1. Schluter D. 2009 Evidence for ecological speciation and its alternative. Science 323, 737–741. (doi:10.1126/science.1160006)
2. Nosil P. 2012 Ecological speciation, p. 280. Oxford, UK: OUP.
3. Hendry AP. 2009 Ecological speciation! Or the lack thereof? Can. J. Fish. Aquat. Sci. 66, 1383–1398. (doi:10.1139/F09-074)
4. Schluter D. 1994 Experimental evidence that competition promotes divergence in adaptive radiation. Science 266, 798–801. (doi:10.1126/science.266.5186.798)
5. Nosil P, Crespi BJ. 2006 Experimental evidence that predation promotes divergence in adaptive radiation. Proc. Natl Acad. Sci. USA 103, 9090–9095. (doi:10.1073/pnas.0601575103)
6. Boughman JW. 2001 Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411, 944–948. (doi:10.1038/sj.nature00824)
7. Seehausen O et al. 2008 Speciation through sensory drive in cichlid fish. Nature 455, U620–U623. (doi:10.1038/nature07285)
8. Haldane JBS. 1949 Disease and evolution. La Ricerca Scientifica 19, 68–76.
9. Summers K, McKeon S, Sellar J, Keusenkothen M, Morris J, Gloeckner D, Pressley C, Price B, Snow H. 2009 Parasitic exploitation as an engine of diversity. Evolution in Mendelian populations of three-spined stickleback, Gasterosteus aculeatus. J. Zool. 199(APR), 535–544.
10. Eizaguirre C, Lenz TL, Traulsen A, Milinski M. 2009 Five questions on the role of enemies in parasitism during the evolution of phenotypic diversity in Outer Hebridean populations of the three-spined stickleback, Gasterosteus aculeatus. J. Evol. Biol. 22, 681–685. (doi:10.1111/j.1420-9101.2008.01627.x)
11. Vamosi SM. 2005 On the role of enemies in parasitism during the evolution of phenotypic diversity in Outer Hebridean populations of the three-spined stickleback, Gasterosteus aculeatus. J. Evol. Biol. 18, 639–675. (doi:10.1111/j.0014-3820.2001.tb01284.x)
12. Thibert-Plante X, Hendry AP. 2009 Five questions on ecological speciation addressed with individual-based simulations. J. Evol. Biol. 22, 109–123. (doi:10.1111/j.1420-9101.2008.01627.x)
13. Yoder JB, Nuismer SL. 2010 When does coevolution promote diversification? Am. Nat. 176, 802–817. (doi:10.1086/657048)
14. Buckling A, Rainey PB. 2002 The role of parasites in sympatric and allopatric host diversification. Nature 420, 496–499. (doi:10.1038/nature01164)
15. Karvonen A, Seehausen O. 2012 The role of parasitism in adaptive radiations—when might parasites promote and when might they constrain ecological speciation? Int. J. Evol. Biol. 2012, 1–20. (doi:10.1152/2012/280769)
16. Nosil P, Vines TH, Funk DJ. 2005 Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59, 705–719. (doi:10.1554/04-428)
17. Scharssack JP, Kalibe M, Harmod C, Rauch G. 2007 Habitat-specific adaptation of immune responses of stickleback (Gasterosteus aculeatus) lake and river ecotypes. Proc. R. Soc. B 274, 1523–1532. (doi:10.1098/rspb.2007.0210)
18. Coyne JA, Orr HA. 2004 Speciation, p. 545. Sunderland, MA: Sinauer Associates.
19. Rundle HD, Whitlock MC. 2001 A genetic interpretation of ecologically dependent isolation. Evolution 55, 198–201. (doi:10.1111/j.0014-3820.2001.tb01284.x)
20. Egan SP, Funk DJ. 2009 Ecologically dependent postmating isolation between sympatric host forms of Neochlamisus bebbianae (Monogenea) leaf and river ectotypes. Proc. Natl Acad. Sci. USA 106, 19 426–19 431. (doi:10.1073/pnas.0907424106)
21. Rundle HD. 2002 A test of ecologically dependent postmating isolation between sympatric sticklebacks. Evolution 56, 322–329. (doi:10.1111/j.0014-3820.2002.tb01342.x)
22. Lynch M, Walsh B. 1998 Genetics and analysis of quantitative traits, p. 980. Sunderland, MA: Sinauer Associates.
23. de Roij J, MacColl ADC. 2012 Consistent differences in macroparasite community composition among populations of three-spined sticklebacks, Gasterosteus aculeatus L. Parasitology 139, 1478–1491. (doi:10.1017/s0031182012000789)
24. MacColl ADC, Chapman SM. 2010 Parasites can cause selection against migrants following dispersal between environments. Funct. Ecol. 24, 847–856. (doi:10.1111/j.1365-2435.2010.01691.x)
25. Giles N. 1983 The possible role of environmental calcium levels during the evolution of phenotypic diversity in Outer Hebridean populations of the three-spined stickleback, Gasterosteus aculeatus. J. Zool. 199(APR), 535–544.
26. MacColl ADC, El Nagar A, de Roij J. 2013 The evolutionary ecology of dwarfism in three-spined sticklebacks. J. Anim. Ecol. 82, 642–652. (doi:10.1111/j.1365-2666.2012.02082.x)
27. Ballantyne CK. 2010 Extent and deglacial chronology of the last British-Irish Ice Sheet: implications of exposure dating using cosmogenic isotopes. J. Quat. Sci. 25, 515–534. (doi:10.1002/jqs.1310)
28. El Nagar A. 2014 The role of parasites in the ecological speciation of three-spined sticklebacks Gasterosteus aculeatus. Nottingham, UK: University of Nottingham.
29. Wright S. 1931 Evolution in Mendelian populations. Genetics 16, 97–159.
30. Chellappa S, Huntingford FA, Strang RHC, Thomson RV. 1995 Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish Biol. 47, 775–787. (doi:10.1111/j.1095-8649.1995.tb06002.x)
31. Sokal RR, Rohlf FJ. 1981 Biometry, 2nd edn, p. 859. New York, NY: W.H. Freeman.
32. Bakke TA, Cable J, Harris PD. 2007 The biology of gyrodactylid monogeneans: the “Russian-doll killers”. Adv. Parasitol. 64, 161–376. (doi:10.1016/S0065-308X(06)6003-7)
33. Scott ME, Anderson RM. 1984 The population dynamics of Gyrodactylus belluslarus (Monogenea) within laboratory populations of the fish host Corella reticulata. Parasitology 89(AUG), 159–194. (doi:10.1017/S0031182000001207)
34. Bakke TA, Mackenzie K. 1993 Comparative susceptibility of native Scottish and Norwegian
stocks of Atlantic salmon, *Salmo salar* L, to *Gyrodactylus salaris* Malmberg—laboratory experiments. *Fish Res.* **17**, 69 – 85. (doi:10.1016/0165-7836(93)90008-u)

35. van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J. 2007 Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int. J. Parasit.* **37**, 805 – 812. (doi:10.1016/j.ijpara.2006.12.016)

36. Pylkko P, Suomalainen LR, Tiinola M, Valtonen ET. 2006 Evidence of enhanced bacterial invasion during *Diplostomum spathaceum* infection in European grayling, *Thymallus thymallus* (L.). *J. Fish Dis.* **29**, 79 – 86. (doi:10.1111/j.1365-2761.2006.00683.x)

37. Karvonen A. 2012 *Diplostomum spathaceum* and related species. In *Fish parasites: pathobiology and protection* (eds PTK Woo, K Buchmann), pp. 260 – 269. Wallingford, UK: CAB International.

38. Michalkova V, Ondrackova M. 2014 Experimental evidence for parasite-induced over-winter mortality in juvenile *Rhodeus amarus*. *J. Fish Biol.* **84**, 1377 – 1388. (doi:10.1111/jfb.12363)

39. Seppanen E, Kuukka H, Voutilainen A, Huuskonen H, Peuhkuri N. 2009 Metabolic depression and spleen and liver enlargement in juvenile Arctic char *Salvelinus alpinus* exposed to chronic parasite infection. *J. Fish Biol.* **74**, 553 – 561. (doi:10.1111/j.1095-8649.2008.02144.x)

40. Eizaguirre C, Lenz TL, Kalbe M, Milinski M. 2012 Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. *Ecol. Lett.* **15**, 723 – 731. (doi:10.1111/j.1461-0248.2012.01791.x)

41. Jones FC, Grabherr MG, Chan YF, Kingsley DM. 2012 The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**, 55 – 61. (doi:10.1038/nature10944)

42. Colosimo FF et al. 2005 Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* **307**, 1928 – 1933. (doi:10.1126/science.1107239)

43. Jones FC et al. 2012 A genome-wide SNP genotyping array reveals patterns of global and repeated species-pair divergence in sticklebacks. *Curr. Biol.* **22**, 83 – 90. (doi:10.1016/j.cub.2011.11.045)

44. Fritz RS, Moula C, Newcombe G. 1999 Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. *Annu. Rev. Ecol. Syst.* **30**, 565 – 591. (doi:10.1146/annurev.ecolsys.30.1.565)