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Experimental evolution of parasite resistance in wild guppies: natural and multifarious selection

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In Dargent *et al*. [1], we reported the evolution of increased resistance in populations of wild female guppies after the removal of an ectoparasite (*Gyrodactylus turnbulli*) and discussed possible mechanisms for this unexpected outcome. In her comment, Stephenson proposes two additional mechanisms that could have led to increased resistance: (i) artificial selection of more resistant individuals both prior to introduction and during laboratory rearing and (ii) differences in *per capita* resource availability due to different guppy densities between source and introduction sites. Additionally, the author suggests that our experimental design was not optimal for testing the effects of parasite removal on the evolution of resistance, because we did not control for some other sources of variation.

Stephenson's suggested alternative mechanisms are certainly valid *a priori* hypotheses that might also occur to other readers. We, therefore, welcome the opportunity to further strengthen our conclusions, first by explaining why these alternatives do not apply to our study, and second by showing how several of the concerns raised by Stephenson support, in a broad sense, a central point of our paper. That is, multifarious selection (i.e. the presence of multiple other variables that could interact with parasitism) is the very context in which selection by parasites should be more often considered. In particular, studies in nature that include these other variables are informative of the conditions under which resistance actually evolves in nature. Resulting insights allow one to assess the importance of parasitism in the evolution of defence when multiple sources of selection, not only parasitism, might matter. We now discuss each of the points raised by Stephenson.

Stephenson suggests that artificial selection could have been imposed by selective mortality due to previous infection, handling and antipathogen treatments. As an example of artificial selection, the author cites a study by van Oosterhout *et al*. [2] that reported 14% handling-induced mortality for wild guppies and 43% handling-induced mortality for laboratory-reared guppies. We agree that 14% mortality is high and could impose unwanted selection. Mortality levels were much lower in our study. Between collection of fish from the source population and release into the introduction streams mortality was less than 2% (A. López-Sepulcre 2014, personal communication). Furthermore, no deleterious effect of antipathogen treatments was detected, marking-induced mortality was below 1% (A. López-Sepulcre & D. Reznick, unpublished data), and fry mortality prior to infection in our laboratory flow-through system was below 3%. These low levels of mortality, even if selective, could not explain our finding of rapid and repeatable evolution of increased resistance to the now-absent parasite.

Stephenson suggested that a temporal pattern of increasing resistance would be one way to substantiate our conclusions by negating concerns regarding artificial selection. As the two collection years were separate experiments, it would have been invalid to directly compare them [1]. However, changes between years/experiments in the magnitude of among-population differences, make the point just as effectively. In particular, the difference between introduced

and source populations increased from the first collection year/experiment to the second, and a population (Lower Lalaja) that was not significantly different from the source population after one year had significantly higher resistance in the second year. This temporal comparison provides independent support (in addition to the above-noted low mortality), removing artificial selection as an explanation for our findings.

Stephenson's second point is that resource availability mediated by guppy density might have contributed to the differences in parasite resistance we observed. We agree that *per capita* resource availability matters, but we did not emphasize this metric as it (in contrast to Stephenson's suggestion) would not be lower in the introduction sites. First, population growth within the introduction streams was rapid because half of the guppies introduced were gravid females, which would have led to at least a fivefold increase in density within the first month. Indeed, by the time our assays were conducted (and probably much earlier), guppy densities were already very similar to those suggested by Stephenson for low-predation sites (table 1; A. López-Sepulcre *et al.*, unpublished data) and did not correlate with infection-assay peak loads ($R^2 = 0.15$, $F_{1,6} = 0.69$, $p = 0.45$). Second, population densities in high-predation sites are generally lower, and productivity higher (owing to more open canopies) than are densities and productivities in low-predation sites [3,4]. As a result, Stephenson's suggestion that *per capita* resource availability would be higher in the low-predation introduction populations than in the high-predation source population is not supported by data for guppies, including our specific populations.

Stephenson's third point is that our study was not optimally designed to detect effects of altered parasitism because other factors differed between the introduction and source populations. This concern certainly would be valid if our goal was to isolate the effects of parasitism from other sources of natural variation, which can be achieved only through laboratory experimental evolution. However, our specific goal was to assess the evolution of parasite resistance in the presence of all other variations, which can be achieved only through experimental evolution *in nature*. Resistance to a common and deleterious parasite is traditionally and theoretically expected to be more strongly influenced by the parasite itself than by correlated factors [5]; yet, as we show, this effect is overridden by other factors (most probably predation) when placed in a natural context. Thus, our study was appropriately designed to explore the questions that we intended to test. In fact, the contrast between Stephenson's concern and our actual conclusions highlights the significance of our study—it specifically tests something that controlled laboratory studies cannot. We agree that disentangling the effects of each axis of variation independently would be an interesting next step having now shown that, under multifarious selection, a pattern expected under univariate selection does not hold.

Interestingly, and as noted in the original paper, our seemingly surprising findings actually mesh quite well with previous findings. In particular, low-predation guppies have lower *Gyrodactylus* loads than do high-predation guppies [6] and previous field introductions from *Gyrodactylus*-present sources (which did not report any anti-parasite treatments), all have no presence of *Gyrodactylus* (exception El Cedro—prevalence 1.4%) [6], which confirms a general higher resistance to parasitism in low-predation populations. Moreover, the high frequency of an MHC allele [7] that correlates negatively with *Gyrodactylus* load [8] is retained in these populations. Even more directly, the historic Turure introduction used an ancestral population from the same site as we did (Guanapo source) over 60 years ago, and these fish remain *Gyrodactylus*-free [6,7]. Similarly, guppies introduced from the Yarra River high-predation site into the previously guppy-free Damier River remained infected in the high-predation reach (prevalence 16%) but not the low-predation reach [6]. Finally, the low-predation population of the Guanapo River (upstream from the ancestral source population but downstream from the introduced populations) was *Gyrodactylus*-free before and during the experiment [6,7]. In short, populations introduced from high- to low-predation sites, either by colonization or by translocation seem to repeatedly evolve increased resistance to *Gyrodactylus* parasites, a pattern inconsistent with traditional ideas but that we were able to experimentally confirm.

We appreciate the comments by Stephenson and the opportunity provided to enhance support for our results by showing that—in our specific study at least—the otherwise valid concerns do not apply. Moreover, they have allowed us to further highlight the importance of considering the role of multifarious selection in natural contexts, such as can only be achieved through experiments in nature. We anticipate that our initially surprising results will continue to spawn alternative explanations, and we hope that these emerging ideas allow the opportunity for further discussion.

Table 1. Guppy densities at the introduction streams. Guppy densities based on mark-recapture models at the introduction streams one and two year after introduction for individuals larger than 14 mm standard length. Data from A. López-Sepulcre *et al.*, unpublished data.

collection	population	density (m^{-2}) (95% CI)
2009, 1 year after introduction	Lower Lalaja	3.41 (2.79–4.02)
	Upper Lalaja	2.40 (1.80–3.01)
2010, 2 years after introduction	Lower Lalaja	8.32 (7.81–8.84)
	Upper Lalaja	9.97 (9.42–10.52)
2010, 1 year after introduction	Taylor	6.36 (5.17–7.56)
	Caigual	4.01 (3.81–4.21)

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