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## Red Queen hypothesis supported by parasitism in sexual and clonal fish

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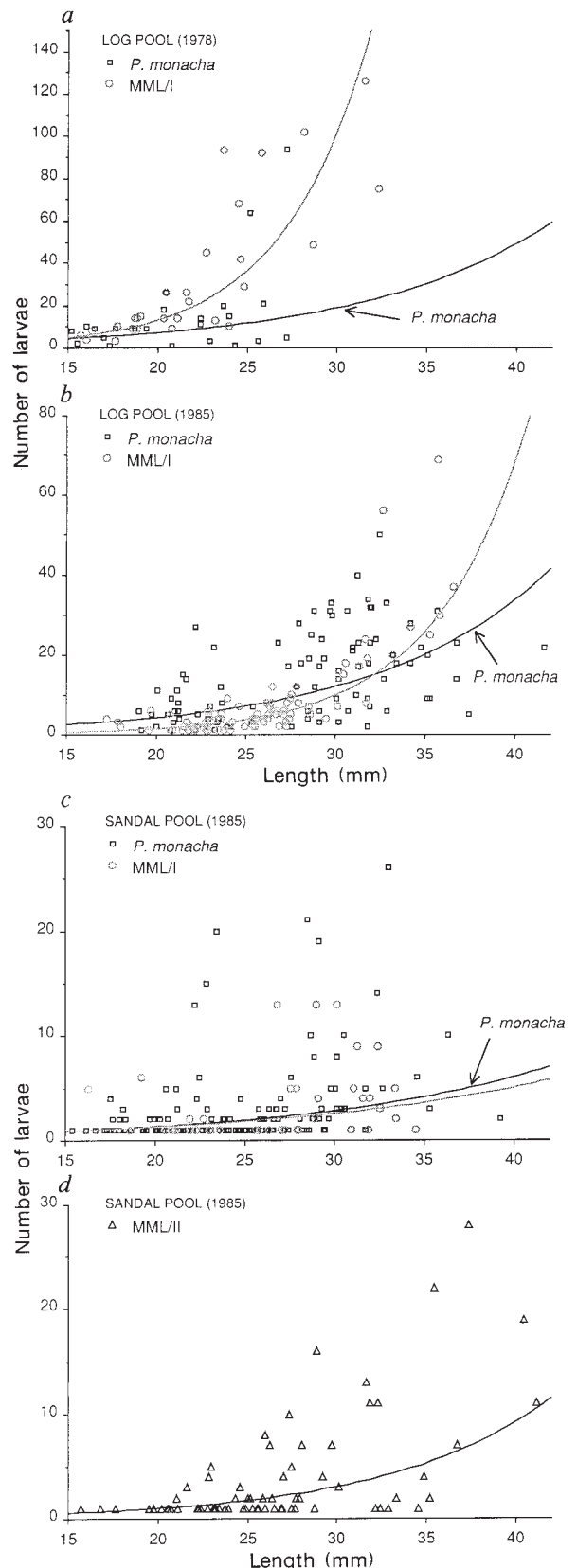
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THE Red Queen hypothesis for the maintenance of biparental sexual reproduction suggests that, for species locked in coevolutionary struggles with biological enemies, the production of variable progeny compensates for the genetic or ecological disadvantages of sex<sup>1-7</sup>. The advantage of sex and recombination under this hypothesis stems from the production of rare phenotypes, which are expected to be more likely to escape infection or predation by coevolved biological enemies. Like many evolutionary hypotheses, the Red Queen hypothesis is difficult to test directly, but its assumptions and predictions can be evaluated<sup>7-18</sup>. The most critical assumption is that biological enemies will disproportionately attack the most common phenotype<sup>19,20</sup>. In this study of parasite loads of coexisting sexual and clonal fish, we find empirical support for this assumption.

The sexual topminnow *Poeciliopsis monacha* (Atheriniformes: Poeciliidae) coexists with two gynogenetic triploid clones of *P. monacha-lucida*, which were formed by hybridization between *P. monacha* and *P. lucida*<sup>21</sup>. The clones are referred to as MML/I and MML/II, and they can be distinguished from each other and from sexual individuals by gel electrophoresis of their proteins and by histocompatibility criteria<sup>22,23</sup>. These fish commonly exhibit black spot disease, an infection by trematode larvae (*Uvulifer* sp.) which form externally visible cysts after burrowing into the body wall<sup>24</sup>. In what follows, we assume that susceptibility to helminth infections of this type is genetically based (reviewed in ref. 25) and that the most common susceptibility phenotype in an outbred sexual population is far less frequent than the most common clone. The Red Queen hypothesis therefore predicts that the more common clone will be more intensely infected than coexisting sexual populations.

We counted the number of cysts infecting fish from three natural rock pools in the Arroyo de las Platanos (Río Fuerte, Sonora, Mexico). The first of these (Log Pool) contains *P. monacha* and MML/I. In the two years in which collections were available for this pool (1978 and 1985), clone MML/I accumulated cysts at a significantly greater rate per unit body length than sexual fish (Fig. 1a and b; Table 1).

A similar result was obtained in the second pool (Sandal Pool, located 274 m below Log Pool), in which sexual *P. monacha* coexist with both MML/I and MML/II. The more common clone in this pool, MML/II, was significantly more parasitized per unit body length than either *P. monacha* or MML/I (Fig. 1c and d; legend to Table 1). We observed no significant difference in the infection levels between the less frequent clone MML/I and *P. monacha* (Table 1), suggesting that the previous result for Log Pool was not an artefact of MML/I being



inherently more susceptible than *P. monacha*, independent of its relative abundance.

In the third pool (Heart Pool, 150 m above Log Pool), a local population extinction and subsequent founder event allowed us to compare parasite loads in MML/I with both homozygous and genetically variable *P. monacha*. Heart Pool dried completely during a severe drought in 1976. The fish recolonized

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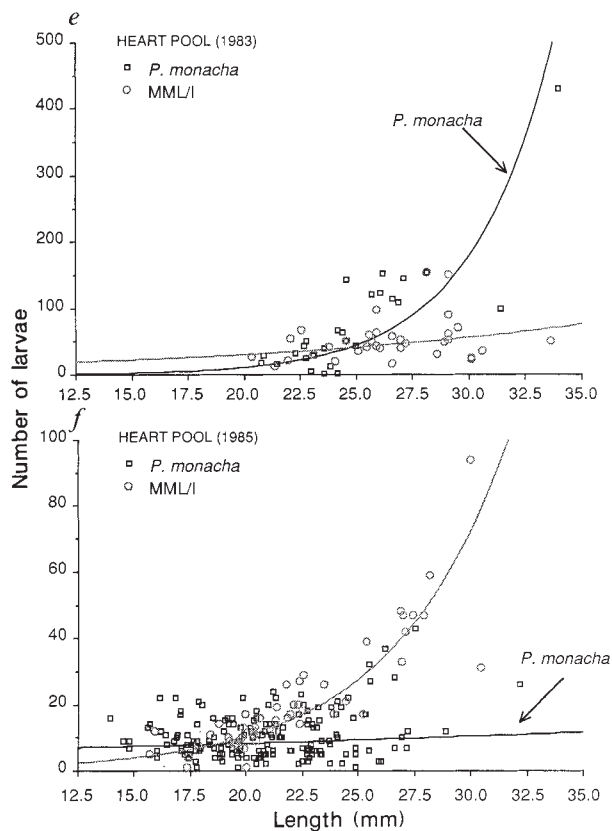


FIG. 1 Bivariate plots of number of encysted trematode larvae (metacercariae) in topminnows against standard length (mm) of the fish. Plots *a* and *b* are for Log Pool for 1978 and 1985. Plots *c* and *d* are for Sandal Pool in 1985: *c* gives the results for MML/I and *P. monacha* in the same form as in the previous plots; *d* gives the results for MML/II. Plots *e* and *f* are for Heart Pool: *e* shows the results for 1983, when the sexual population was highly inbred (removing the 'outlier' of over 400 parasites in this plot does not alter the conclusion that the two slopes are significantly different); *f* shows the results for 1985, two years after the introduction of sexual females from the Jaguari river. The formal statistical analysis is given in Table 1. The curves approximate the best fit line from the back-transformed log-linear plots.

the pool by 1978, but genotypic diversity in the inbred sexual founders was severely reduced (changes in gene diversity and population dynamics of these populations are discussed in refs 26 and 27). In 1983, genetically variable sexual females were transplanted from a nearby downstream population, resulting in an increase in the local genotypic diversity of the sexual population<sup>26</sup>. Collections of the fish from Heart Pool were made both before (1983) and after (1985) the transplant.

Before the transfer of sexual females into Heart Pool, the number of parasites per unit length of fish was significantly greater in the highly homozygous inbred sexual fish than in clone MML/I (Fig. 1*e*; Table 1). Because MML/I was more common than sexual *P. monacha*, this result is inconsistent with the prediction of greater infection of the most common phenotype. But the higher infection of the inbred sexual individuals might have been due to the detrimental effects of inbreeding depression; inbreeding in this pool resulted in a decline of developmental stability and other fitness-related traits<sup>26,27</sup>. Two years after the transfer of sexual females to Heart Pool, the trend had reversed and the number of parasites per unit length of fish was significantly greater in clonal fish than in the outbred sexual fish (Fig. 1*f*; Table 1).

If overall levels of genotypic variability are correlated with variance in disease susceptibility, outbred sexual individuals should exhibit greater variance in parasite loads than both the

TABLE 1 Analysis of covariance summary

Contrast	Pool	Year	Mean sum of squares		F	P
			Interaction (d.f.)	Error (d.f.)		
1	Log	1978	1.21 (1)	0.201 (48)	6.02	0.018
2		1985	1.14 (1)	0.114 (171)	10.06	0.002
3	Heart	1983	3.81 (1)	0.291 (57)	13.08	<0.001
4		1985	5.31 (1)	0.094 (215)	56.60	<0.001
5	Sandal	1985	0.17 (2)	0.054 (251)	3.20	0.042

Analysis of covariance summary table for the heterogeneity of slopes model<sup>29</sup>. The dependent variable, number of trematode cysts in the body wall, was log-transformed<sup>30</sup> before the analysis:  $n' = \log(n + 10)$ . The independent variables were reproductive mode (sexual versus asexual) and standard length of the fish (covariate). A significant *F* value in the table indicates an interaction between reproductive mode and standard length, meaning the slopes of the regression lines for parasite number against length are significantly different. We interpret significant differences to reflect differences in the rates of accumulation of parasites as a function of age of the fish. We feel that larger fish show more of a difference between strains because they have had more time to accumulate their parasite loads. (Because both clones are pseudogamous, we do not intend to imply from this analysis that the trematode is selecting for the maintenance of sex in these fish.) The comparisons were between *P. monacha* and MML/I in all cases except Sandal Pool, wherein *P. monacha*, MML/I, and MML/II were compared. For Sandal Pool, the more common clone, MML/II, had a significantly greater slope than *P. monacha* and MML/I ( $F_{1,253} = 6.57$ ;  $P = 0.011$ ); the difference between *P. monacha* and MML/I was not significant ( $F_{1,178} = 0.21$ ;  $P = 0.648$ ). Because the assumption of homoscedasticity was not met in contrasts 1, 2 and 4 (see Table 2), the probability values given here are only approximate.

TABLE 2 Variance ratios

Pool	Year	Residual variance		F ratio	P
		Numerator (d.f.)	Denominator (d.f.)		
Log	1978	0.051 (23)	0.025 (28)	2.04	<0.050
	1985	0.029 (90)	0.012 (80)	2.42	<0.001
Heart	1983	0.078 (26)	0.084 (33)	0.93	0.571
	1985	0.020 (170)	0.010 (47)	2.00	<0.005
Sandal	1985				
	<i>P. monacha</i> , MML/I	0.010 (137)	0.010 (43)	1.00	0.483
	<i>P. monacha</i> , MML/II	0.010 (137)	0.011 (73)	0.91	0.307
	MML/I, MML/II	0.010 (73)	0.011 (43)	0.91	0.341

The residual variance for the sexual population divided by the residual variance for MML/I (*F* ratio), except in Sandal Pool as indicated. The residuals were calculated from the regression of the number of cysts (transformed to  $\log(\text{cysts} + 10)$ ) against standard length of the fish. Significant differences imply a greater genetic variation for susceptibility to infection in the sexual subpopulation. Such differences, however, are also in violation of the homogeneity of variance assumption for the ANCOVA in Table 1, to which the analysis is normally considered to be robust<sup>29</sup>.

inbred founder individuals and the genetically uniform clones. We examined this hypothesis by comparing the residual variances in parasite loads (Table 2). For both of the Log Pool samples and the 1985 Heart Pool sample, genetically variable sexual fish exhibited twice the variance in infection levels of the coexisting clone (a highly significant ratio, see Table 2). The homozygous founder population of *P. monacha* from Heart Pool in 1983 did not differ significantly from the clone in this regard. Hence, the greater variation in parasite loads in sexual fish seems to be associated with outbreeding. The parasite load of genotypically variable sexual individuals from Sandal Pool was not significantly more variable than those of the coexisting clonal forms. The reasons for this discrepancy with the other pools are unknown but might be due to the low levels of infection in Sandal Pool (Fig. 1).

In summary, the *Poeciliopsis* system provided an opportunity to examine the assumptions of the Red Queen hypothesis in a natural ecological setting, because it represents a rare situation where clonal and non-clonal forms are recognizable and coexist. We found that the more common clone accumulated parasites at a significantly greater rate than the sexual subpopulation, except in the case where the sexual subpopulation was highly inbred following a founder event. This result corroborates agriculture's experience with monocultures<sup>28</sup> and supports an important assumption of the Red Queen hypothesis: parasites

evolve to disproportionately infect genetically uniform strains as they become common. The results are also consistent with our assumption of a genetic basis to the host-parasite interaction, and with the expectation that sexual reproduction provides a partial escape from biological enemies only in genotypically diverse populations. □

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## A cholecystokinin-like hormone activates a feeding-related neural circuit in lobster

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**THE peptide hormone cholecystokinin (CCK) contributes to the production of feeding-related behaviour in mammals, but the mechanism by which it exerts its effects remains unclear<sup>1–6</sup>. The gastric mill neural circuit of lobster is an experimentally accessible model system for studying the hormonal control of feeding-related behaviour<sup>7,8</sup>. Composed of 11 identified neurons, this circuit produces rhythmic movement of teeth within the stomach<sup>9</sup>. We have previously shown that the gastric mill motor pattern can be modulated by a cholecystokinin-like peptide *in vitro*<sup>10</sup>. We report here that (1) after feeding, levels of CCK-like peptide in haemolymph increase with the activation of the gastric mill, (2) injections of CCK activate the gastric mill, and (3) a specific CCK antagonist inhibits feeding-induced gastric mill activity. This neatly demonstrates a causal link between *in vivo* release of a peptide hormone and activation of a neural circuit.**

In several vertebrates and invertebrates, blood levels of CCK-like peptide (CCKLP) rise after feeding<sup>11–14</sup>. We compared changes in blood CCKLP levels in the lobster *Panulirus interruptus* with the activation of the gastric mill (GM) following feeding. GM activity was monitored in freely moving lobsters by recording the activity of the gm1 muscle, which protracts the medial tooth of the GM. In individual experiments, the GM

began cycling immediately after feeding, cycled continuously for 2–6 h, then the burst strength and frequency declined and activity became intermittent; this intermittent activity could continue for up to 48 h (Fig. 1a). On average, the GM reached its peak frequency during the first hour after feeding; the average frequency then declined over the next 3 h, but remained above base line for the next 4 h (Fig. 1b).

CCK-like peptide is found in lobster neurohaemal organs and haemolymph (G.G.T. and A.I.S., manuscript submitted). Here we use a radioimmunoassay to monitor the levels of CCKLP in haemolymph before and after feeding. There was a roughly fourfold increase in the levels of CCKLP in the first hour after feeding, peaking at an average of  $(1.6 \pm 0.5) \times 10^{-9}$  M, and levels

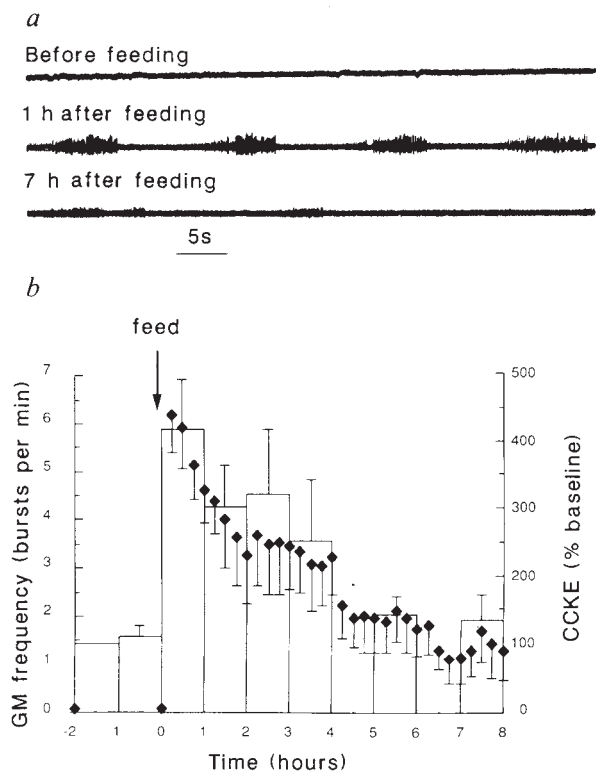


FIG. 1 Activity of the GM, and levels of CCKLP in haemolymph, before and after feeding. *a*, Gm1 muscle activity in one animal before feeding, at 1 h after feeding, and at 7 h after feeding. Before feeding there is no GM activity. At 1 h, the GM is cycling regularly, with a period of  $\sim 10$  s. At 7 h the GM is cycling intermittently, and the individual muscle bursts are weaker than at 1 h. *b*, Average GM activity and average CCKLP levels in the haemolymph before and after feeding. Diamonds, average GM frequency, mean  $\pm$  s.e.m. ( $n=6$ ). Histograms, average CCKLP levels in haemolymph, mean  $\pm$  s.e.m. ( $n=7$ ); levels were significantly raised for the first 2 h after feeding ( $P < 0.01$ , Student's *t*-test).

**METHODS.** Myograms: Lobsters were starved for 1–3 weeks, kept on an artificial light–dark cycle, and fed  $\sim 10$  g smelt or squid in the dark. Myograms were obtained from unrestrained animals by implanting teflon-coated silver wire electrodes into the gm1 muscle; electrode placement was verified at the end of the experiment<sup>30</sup>. Animals recovered 2–7 days before initiation of experiments. Signals were differentially amplified, recorded on videocassette, and displayed with a Gould ES1000 electrostatic recorder. Generally 16 h of activity were recorded; activity was then monitored intermittently for up to 36 h. Muscle spikes were converted into constant-voltage pulses using a Schmidt trigger; pulses were digitized and analysed using an IBM PC. Bursts were defined as more than 20 spikes less than 1 s apart. Data were averaged over 15-min time blocks; values are mean  $\pm$  s.e.m. CCKLP levels: Lobsters were maintained and fed as above. Aliquots (1 ml) of haemolymph were withdrawn from the ventral artery, extracted with ethanol, microfuged, the supernatants dried in a Speed-Vac concentrator, then reconstituted in buffer and assayed in duplicate by radioimmunoassay as described<sup>10</sup>, values are expressed as CCK molar equivalents (CCKE). Several haemolymph samples were obtained before feeding, and at  $\sim 1$  h intervals thereafter. Data were normalized to first baseline value, and averaged over 1 h time blocks.