A comparison of parasite loads on asexual and sexual *Phoxinus* (Pisces: Cyprinidae)

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Abstract: In light of the inherent disadvantages of sexual reproduction, the existence of sex is often seen as a paradox. There are a variety of hypothetical benefits of sexual reproduction that may balance its disadvantages. The Red Queen hypothesis proposes that sexually reproducing species are better able to evolve resistance to parasites than asexually reproducing species. A prediction of the Red Queen hypothesis is that a parasite should evolve to preferentially exploit an asexual species over a sexual species. To test this central prediction of the Red Queen hypothesis, intensity of infection by the parasite *Gyrodactylus eos* Mayes, 1977 (Monogenea) was compared between sympatric asexual and sexual fish species in the genus *Phoxinus* Rafinesque, 1820. In each lake where these species coexist, the asexual fish should suffer higher intensities of infection than the sexual fish. In the majority of lakes sampled, there were more parasites on asexual than sexual fish.

Résumé : À cause des désavantages inhérents à la reproduction sexuée, l'existence de la sexualité apparaît souvent paradoxale. Il y a cependant une gamme d'avantages hypothétiques à la reproduction sexuée qui peuvent compenser les désavantages. L'hypothèse de la reine rouge veut que les espèces à reproduction sexuée soient plus aptes à développer une résistance aux parasites que les espèces à reproduction asexuée. Une des prédictions de l'hypothèse de la reine rouge est qu'un parasite devrait s'adapter à exploiter de préférence une espèce asexuée plutôt qu'une espèce sexuée. Pour vérifier cette prédiction clé de l'hypothèse de la reine rouge, nous avons comparé l'intensité de l'infection par le parasite *Gyrodactylus eos* Mayes, 1977 (Monogena) chez des espèces de poissons sympatriques, l'une sexuée et l'autre asexuée, du genre *Phoxinus* Rafinesque, 1820. Dans tous les lacs où les espèces coexistent, les poissons asexués devraient avoir des intensités d'infection plus importantes que les poissons sexués. Dans la majorité des lacs échantillonnés, il y a plus de parasites chez les poissons asexués que chez les sexués.

[Traduit par la Rédaction]

Introduction

No one knows for sure why sexual reproduction is nearly ubiquitous among the extant species of the globe. Given a 50% reduction in reproductive rate and a variety of other fitness costs associated with sexual reproduction relative to asexual reproduction (Williams 1975; Barash 1976; Maynard Smith 1978; Charlesworth 1980; Hastings 1999), it is quite remarkable that sex has become, and manages to remain, so dominant. Clearly, there must be some advantage, or variety of advantages, conferred by sexual reproduction. Elucidating the potential benefits of sexual reproduction is an ongoing struggle dating back at least to Darwin (1862): "We do not even in the least know the final cause of sexuality; why new beings should be produced by the union of the two sexual elements, instead of by parthenogenesis . . . The whole subject is as yet hidden in darkness." There have been numerous attempts to provide a widely accepted theory of sex (e.g., Fisher 1930; Muller 1964; Crow and Kimura 1965; Williams 1975; Maynard Smith 1976, 1978; Hamilton 1980; Bell 1982; Kondrashov 1982, 1988; Charlesworth 1990; Roughgarden 1991; e.g., Barton 1995; Agrawal 2001; Otto and Barton 2001; Siller 2001), but none have succeeded, mainly because of a lack of empirical evidence. Indeed, Bell (1982) saw fit to pronounce that "sex is the queen of problems in evolutionary biology."

The *Phoxinus* Rafinesque, 1820 species complex is one of many vertebrate species complexes that includes an asexual species of hybrid origin (New 1962). Asexual *Phoxinus* populations are distributed throughout most of Canada and the northern United States, and coexist with either or both of the progenitor species (*P. eos* (Cope, 1861) and *P. neogaeus* Cope, 1867) (Goddard et al. 1989). These asexuals, which are almost all female, reproduce via gynogenesis (Goddard et al. 1998). In contrast with many other asexual species (e.g., Vrijenhoek et al. 1978; Lively and Dybdahl 2000), there is very little, if any, genetic variation within or even among diploid asexual *Phoxinus* populations (Elder and Schlosser 1995; Goddard et al. 1998). Also, Goddard et al. (1998) showed that hybrids born of a captive population in 1989 were genetically identical to a hybrid born of the same population in 1986. The temporal and spatial genetic uniformity found in and among asexual *Phoxinus* populations suggests that these populations contain a single, self-perpetuating, diploid clonal lineage and that they do not rely on repeated hybridization to persist. This clonal uniformity also suggests that the asexual genome is non-segregating, evolves slowly, and is the most common genotype where it exists.

There is no lack of hypothetical benefits to sex (Kondrashov 1993), but consensus on the importance of any one hy-

Received 14 December 2005. Accepted 28 April 2006. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 11 July 2006.

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doi:10.1139/Z06-064

pothesis is lacking. One hypothesis that has garnered some interest and is amenable to comparative tests is known as the Red Queen hypothesis (Vanvalen 1973; Jaenike 1978; Hamilton 1980; Bell 1982). According to the Red Queen, sex provides an advantage to hosts by allowing the creation or re-creation of rare genotypes that confer resistance to parasites. A sufficiently virulent parasite will induce an antagonistic coevolutionary process where the host cycles through genes that confer resistance and the parasite cycles through genes that confer infectivity. A sexual host can rapidly create or recreate resistance gene combinations through segregation and recombination, while an asexual host cannot.

The Red Queen hypothesis has been tested in many systems, some providing evidence supporting the Red Queen (e.g., Lively et al. 1990; Moritz et al. 1991; Lively 1999; Hakoyama et al. 2001) and others contradictory to the Red Queen (e.g., Brown et al. 1995; Hanley et al. 1995; Weeks 1996; Tobler et al. 2005). Assessing the general importance of the Red Queen as an explanation for why sex is advantageous requires that more systems be examined where related sexual and asexual species can be compared. In the case of *Phoxinus*, the Red Queen predicts that, since the clonal asexual genome is always the most common genome in a given lake and since this genome has, ostensibly, remained unchanged for many generations, wherever asexual and sexual *Phoxinus* exist in sympatry the asexual individuals will harbour more parasites than the sexual individuals. This prediction follows from the theory developed by Hamilton (1980; 1990).

In this study, the intensity and prevalence of infection by a monogenean ectoparasite, *Gyrodactylus eos* Mayes, 1977 (Monogenea), was compared between sexual and asexual fish in the genus *Phoxinus* from lakes in Algonquin Park, Ontario. It is important to note that the effectiveness of parasites in providing an advantage to a sexual host is lessened if there is variation among individuals in an asexual host population. Such is the case in studies by Lively (1987), Lively et al. (1990), and Moritz et al. (1991) which compare parasitism in a sexual host to that in a genetically variable asexual host. The lack of variation among asexual *Phoxinus*, therefore, means that it is a not unreasonable to predict that the Red Queen should be effective in this system. Empirical evidence for the maintenance of sex by parasites is rare. If there are more parasites on asexual than sexual *Phoxinus*, then a condition for a role of parasites in contributing to the advantage of sex in this system will have been met.

Methods

Algonquin Park, Ontario, is located 300 km north of Toronto and encompasses highlands and headwaters associated with a number of major drainage basins. Much of the park has been protected or managed as a park for over 100 years. As such, lakes within Algonquin Park are generally pristine, although a variety of game fish species have been introduced to the park, and park managers routinely stock some lakes. There are, currently, strict regulations prohibiting and preventing the release or use of bait fish in the park. Some communities of *Phoxinus* (common bait fish) sampled in this study, especially those in easily accessed lakes, were likely affected by unnatural gene flow prior to the enactment of regulations governing the release of bait fish. It is unclear, however, how unnatural gene flow in the past might have affected the Algonquin Park *Phoxinus* communities, and hence the interpretation of the results of this study, and this topic is not discussed further in this paper.

During the summer of 2004, 17 lakes in Algonquin Park that contained *Phoxinus* hybrids were sampled. Most of the samples came from small boggy lakes with abundant beaver lodges, and many were at some stage of succession following beaver-dam construction. Lakes were sampled using 0.5 cm mesh minnow traps baited with Purina Dog Chow and set overnight in littoral regions, as prescribed by He and Lodge (1990). Preliminary studies in 2003 revealed that intensity and prevalence of *G. eos* infection on *Phoxinus* species peaked around the 20th of June and declined to nearly zero by the beginning of August. Therefore, sampling in 2004 was concentrated in mid-June to increase the number of samples with a prevalence of infection high enough to allow a comparison of infection between asexual and sexual *Phoxinus*.

At least 20 *Phoxinus* from each lake were dissected and examined under a dissecting microscope to identify each fish to species and to determine the intensity of *G. eos* infection on each fish. Fish were killed by immersion in a clove oil solution (approximately 500 ppm) prior to examination. Species were identified using a combination of internal and external morphology, as prescribed in Goddard et al. (1989). The most reliable morphological difference between the species is the number of pharyngeal teeth. *Phoxinus eos* has a single row of pharyngeal teeth, *P. neogaeus* has a second outer row with two teeth, and hybrid *Phoxinus* has only one tooth in the outer row of pharyngeal teeth. Less than 1% of species identifications based on morphology have been shown to be erroneous (Schlosser et al. 1998). The intensity of *G. eos* infection on each fish was estimated by counting the parasites on all fin surfaces. The parasites tend to be found almost entirely on fins as opposed to other body surfaces, save for in the highest intensity infections (J.A. Mee, personal observation).

Because of the seasonal nature of *G. eos* infection and because each lake was sampled on a different date, there should be no expectation that prevalence or intensity of infection would correlate with frequency of sexual reproduction across lakes. Instead we predicted that on any given date in each lake where the asexual and sexual species coexist (i.e., in any given sample) the asexual species should harbour more parasites than the sexual species. This prediction lends itself to a comparison of species pairs in each sample. The Wilcoxon signed-rank test was used to compare intensity of infection between asexual and sexual *Phoxinus* populations. It should be noted that, since *P. neogaeus* is an uncommon member of *Phoxinus* communities (Doeringsfeld et al. 2004) and *G. eos* rarely infects *P. neogaeus* (Table 1), all analyses included only hybrids and *P. eos*.

In the calculation of the Wilcoxon z ratio, we included a –0.5 correction for continuity. The median intensity of infection was used in all comparisons of infection intensity, as the median is more representative than the mean in aggregated parasite distributions (Neuhauser and Poulin 2004). For the four lakes that were sampled more than once, the weighted average of median infection intensity, weighted by sample size, was used for the analyses.

Table 1. Infection and length data for all *Phoxinus* species in each sample of each lake sampled in 2004.

					Intensity of infection			
Lake name	Sampling date	Species	*n*	Prevalence of infection (%)	Mean	Median	Adjusted median	Mean length (cm)
Cauliflower	3 June 2004	*P. eos*	2 (0)	100 (na)	13 (na)	13 (na)	13 (na)	4.37 (na)
		Hybrid	2	100	30.5	30.5	28.45 (na)	5.75
Chibiabos	1 July 2004	*P. eos*	22 (15)	86 (86)	4.95 (4.27)	3.5 (3)	3.5 (3)	4.02 (4.08)
		Hybrid	2	100	15	15	14.52 (14.63)	4.98
		P. neogaeus	3 (1)	0 (0)	0 (0)	0 (0)	—	4.79 (5.46)
Daisy	9 June 2004	*P. eos*	5 (4)	100 (100)	31.4 (32.25)	28 (32)	28 (32)	4.69 (4.84)
		Hybrid	9	100	44	41	40.08 (40.48)	5.82
Eos	18 May 2004	*P. eos*	16 (9)	75 (67)	3 (2.3)	2 (2.5)	2 (2.5)	4.38 (4.52)
		Hybrid	2	100	6.0	6	5.99 (5.99)	4.63
		P. neogaeus	1 (1)	0 (0)	0 (0)	0 (0)	—	4.53 (4.53)
	18 June 2004	*P. eos*	15 (7)	93 (86)	7.4 (5.14)	5 (5)	5 (5)	4.07 (4.31)
		Hybrid	2	100	14.5	14.5	14.19 (14.41)	4.93
		P. neogaeus	2 (1)	50 (100)	0.5 (1)	0.5 (1)		4.98 (5.46)
Eucalia	7 May 2004	*P. eos*	14 (6)	21 (50)	0.93 (2.2)	0 (0.5)	0 (0.5)	4.15 (4.41)
		Hybrid	1	100	4.0	4	3.07 (3.66)	5.29
		P. neogaeus	5 (2)	29 (50)	0.8 (0.5)	0 (0)	—	4.63 (4.44)
	18 May 2004	*P. eos*	14 (7)	50 (43)	7.36 (11.57)	0.5 (0)	0.5 (0)	4.13 (4.35)
		Hybrid	6	100	8.17	8	7.92 (7.98)	4.75
	6 June 2004	*P. eos*	26 (10)	76 (70)	3.06 (3.5)	2 (1.5)	2 (1.5)	4.33 (4.55)
		Hybrid	3	100	17.3	9	8.99 (8.99)	4.70
		P. neogaeus	4 (4)	50 (50)	11 (11)	0.5 (0.5)	—	5.08 (5.08)
	18 June 2004	*P. eos*	14 (9)	100 (100)	9.64 (4.7)	6 (3)	6 (3)	4.14 (4.25)
		Hybrid	2	100	2.0	2	1.95 (1.98)	4.67
		P. neogaeus	1 (1)	0 (0)	0 (0)	0 (0)	—	3.55 (3.55)
Hambone	10 June 2004	*P. eos*	15 (9)	93 (100)	24.13 (17.78)	19 (18)	19 (18)	5.04 (5.18)
		Hybrid	3	80	16.67	21	20.92 (20.97)	5.66
Indian Pipe	1 July 2004	*P. eos*	23 (15)	96 (93)	4.13 (3.67)	3 (3)	3 (3)	4.09 (4.19)
		Hybrid	5	100	4.4	3	2.91 (2.95)	4.73
Little Eagle	9 June 2004	*P. eos*	11 (6)	64 (33)	3.64 (0.67)	2 (0)	2 (0)	4.36 (4.43)
		Hybrid	10	70	2.6	1	0.90 (0.93)	5.01
Pezheki	13 June 2004	*P. eos*	11 (11)	100 (100)	13.64 (13.64)	6 (6)	6 (6)	5.73 (5.73)
		Hybrid	12	92	16.75	5	5.00 (5.00)	5.54
Pondweed	17 June 2004	*P. eos*	24 (15)	83 (73)	4.46 (3.2)	3 (2)	3 (2)	4.04 (4.14)
		Hybrid	7	86	7	4	3.60 (3.74)	4.96
Rod and Gun	16 June 2004	*P. eos*	8 (3)	100 (100)	21.25 (13)	15 (13)	15 (13)	4.79 (5.30)
		Hybrid	13	100	34.62	26	25.67 (25.98)	5.66
Raven	16 May 2004	*P. eos*	3 (0)	33 (na)	0.67 (na)	0 (na)	0 (na)	4.82 (na)
		Hybrid	3	100	24.7	4	4.00 (na)	4.56
	6 July 2004	*P. eos*	24 (8)	92 (88)	6.29 (4.38)	5.5 (3)	5.5 (3)	4.22 (4.10)
		Hybrid	6	100	50.83	8	8.00 (8.00)	4.09

Table 1 (*concluded*).

Lake name	Sampling date	Species	*n*	Prevalence of infection (%)	Intensity of infection			Mean length (cm)
					Mean	Median	Adjusted median	
Rence	4 July 2004	*P. eos*	26 (11)	96 (91)	8.38 (4.73)	5.5 (4)	5.5 (4)	4.28 (4.45)
		Hybrid	2	100	9.5	9.5	9.43 (9.48)	4.88
		P. neogaeus	1 (0)	100 (na)	4 (na)	4 (na)	—	4.63 (na)
Ringneck	6 June 2004	*P. eos*	16 (4)	89 (75)	9.89 (8.0)	7 (8.5)	7 (8.5)	4.21 (4.23)
		Hybrid	1	100	1.0	1	1.00 (1.00)	4.21
	3 July 2004	*P. eos*	23 (13)	43 (54)	1.09 (1.54)	0 (1)	0 (1)	4.29 (4.59)
		Hybrid	4	50	3.75	1	0.34 (0.82)	5.33
		P. neogaeus	3 (1)	33 (0)	0.33 (0)	0 (0)	—	4.96 (5.13)
Sunday Creek	2 June 2004	*P. eos*	16 (7)	88 (71)	3.06 (3.14)	3 (3)	3 (3)	4.58 (4.89)
		Hybrid	12	50	6.25	1	1.00 (1.00)	4.71
Sitting Duck	2 June 2004	*P. eos*	18 (13)	50 (46)	1.17 (1.31)	0.5 (0)	0.5 (0)	4.02 (4.14)
		Hybrid	2	100	21.5	21.5	21.33 (21.41)	4.76
		P. neogaeus	1 (1)	0 (0)	0 (0)	0 (0)	—	3.78 (3.78)
Welcome	5 July 2004	*P. eos*	27 (5)	100 (100)	17.85 (19.4)	15 (14)	15 (14)	4.29 (4.38)
		Hybrid	3	100	16.33	11	9.92 (10.20)	5.47

Note: Values in parentheses exclude males. Adjusted median is the median infection intensity adjusted using the length–intensity relationship from data collected in 2005 (see Methods; Fig. 1).

The standard length (the length of a fish measured from the tip of the snout to the posterior of the caudal peduncle) of each fish was also recorded. If fish length were to differ between species, then a significant difference between species in infection intensity, should such a difference exist, may be due to the effect of length and not species. The greater surface area of larger fish may allow greater infection intensities and lead to high transmission rates. A general linear model was constructed (JMP® version 4.0.2; SAS Institute Inc. 2000) to compare length between species and among lakes.

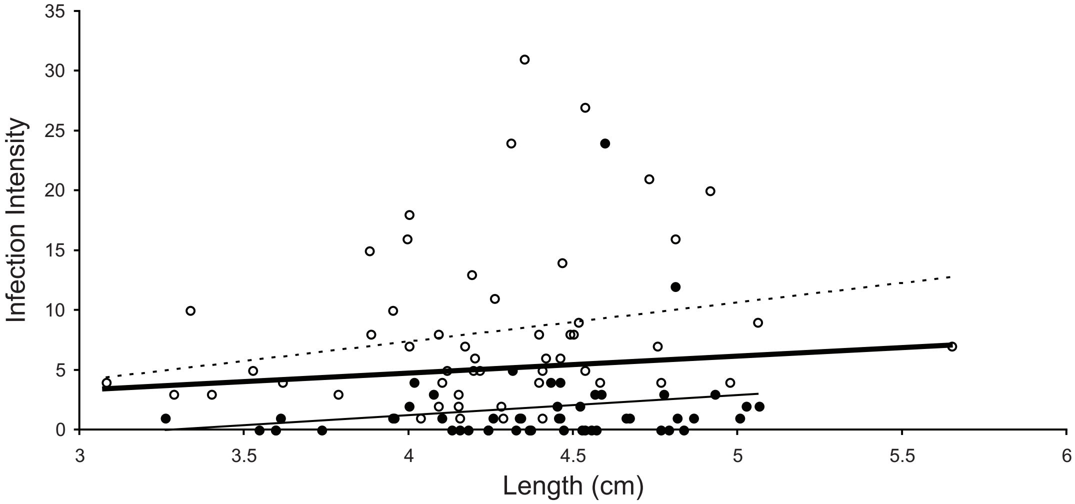
The *Phoxinus* samples collected in 2004 often contained fewer individuals of either species than were necessary to allow a meaningful interpretation of the correlation coefficient for the relationship between length and infection intensity, and in most cases there was no significant correlation. Also, the relationship between infection intensity and length varied between species, among lakes, and among repeated samples of the same lake (the slope was significantly different from zero in only 5 out of 23 samples of *P. eos* and ranged –7.37 to 30.05). As a result, adjusting infection intensities using the 2004 parasitism and length data in a general linear model, with reproductive mode and lake as factors and fish size as a covariate, was unadvisable. Therefore, we returned to Algonquin Park in 2005 to collect large enough samples from two lakes, Lake Eos ($n = 53$) and Lake Eucalia ($n = 50$), to allow a meaningful estimate of the effect of length on infection intensity. Admittedly, however, correcting for size in 2004 samples using 2005 data are not ideal.

Since collecting large samples of asexual *Phoxinus* is a relatively difficult task and since we wished to avoid the influence of gender when calculating the effect of size on intensity of infection, only female *P. eos* were collected in 2005. We constructed a general linear model (JMP® version 4.0.2; SAS Institute Inc. 2000) using the 2005 data to determine how intensity of infection was influenced by length, lake, and a length-by-lake interaction. A square-root transformation was used to bring the negative binomial distribution of infection intensities closer to a normal distribution. Since infection intensity is most likely influenced by surface area rather than length, per se, the square of length was used in our model. We then used our model, constructed using the 2005 data, to modify the median intensity of infection in each sample collected in 2004. This modification was based on the difference in mean length between the pair in each sample.

Results

Among the samples collected in 2004 (Table 1), asexual fish were larger than sexual fish ($F = 50.9$, $p < 0.0001$), females were larger than males ($F = 37.6$, $p < 0.0001$), and there was significant variation in fish size among lakes ($F = 16.4$, $p < 0.0001$). Based on the samples collected in 2005 (Fig. 1), larger fish tended to harbour more parasites than smaller fish ($F = 5.76$, $p = 0.0183$), there was a significant effect of lake on infection intensity ($F = 73.9$, $p < 0.0001$), but there was no length-by-lake interaction ($F = 0.0078$, $p = 0.929$). The scaled estimate of the slope for the relationship between the square root of infection intensity and the square of length (slope = 0.750) from the

Fig. 1. Infection intensity of *Gyrodactylus eos* relative to fish length for female *Phoxinus eos* collected from Eos Lake (○) and Lake Eucalia (●) in 2005. The dashed line is the regression line for Lake Eucalia, the thin solid line is the regression line for Lake Eos, and the thick solid line is the regression line for both lakes combined.



2005 data was used to correct for differences in length when comparing intensity of infection between species or genders for the samples collected in 2004.

Median infection intensities were significantly higher for male *P. eos* than female *P. eos* ($n = 16$; Wilcoxon $z = 2.71$, $p < 0.005$). This difference in infection intensities between the sexes was likely due to morphological differences between the sexes. Male pectoral fins have thicker fin rays and deeper folds between fin rays than female pectoral fins. These features of the male pectoral fin seem to provide a secure microhabitat for gyrodactylid attachment that is more protected from abrasion than any site on the female pectoral fin. The possibility that a sexually dimorphic fin morphology trait can cause a difference between the sexes in infection intensity suggests that only the female infection intensities in *P. eos* populations should be compared with infection intensities in the asexual *Phoxinus* populations. Therefore, comparisons were made between the species both including and excluding males (see Table 1, Fig. 2). Asexual *Phoxinus* had significantly higher median infection intensities than sexual *Phoxinus* whether or not males were included in the analysis (with males: $n = 17$, Wilcoxon $z = 2.09$, $p < 0.025$; without males: $n = 16$, Wilcoxon $z = 2.41$, $p < 0.01$).

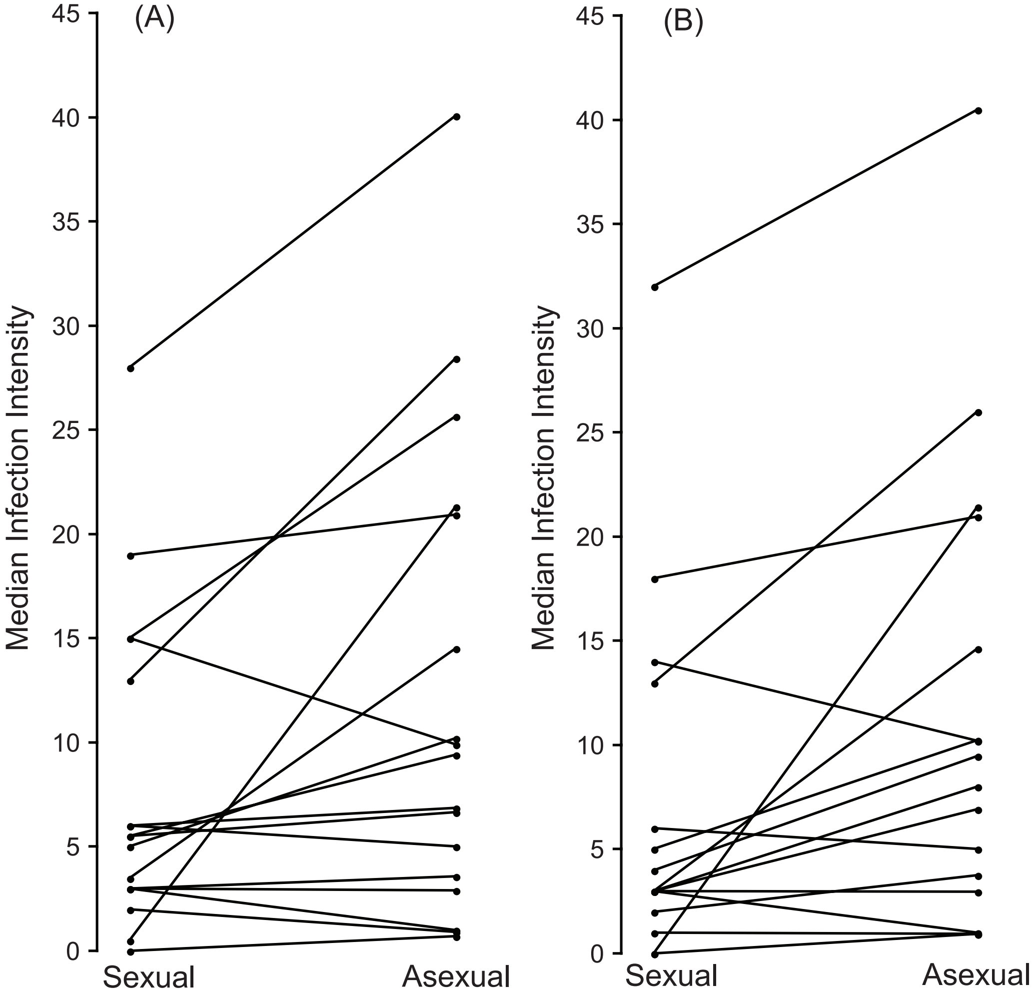
Discussion

The Red Queen hypothesis predicts that the asexual species should harbour more parasites than the sexual species in each lake where asexual and sexual *Phoxinus* coexist. There were, in fact, more *G. eos* on asexual than on sexual *Phoxinus* sampled in Algonquin Park in 2004. This result lends support to the role of parasites in providing an advantage to sex in the *Phoxinus* species complex.

The explanatory power of the Red Queen hypothesis is, however, an increasing function of the pathogenicity of the parasite (May and Anderson 1983). More pathogenic parasites will have a greater role in providing an advantage to sex. There is a good possibility that *G. eos*, like other gyrodactylids (Cone and Odense 1984; Lyles 1990), has at least some pathogenic effect. Individuals with high infection intensities (i.e., greater than 50 parasites on a fish) were, however, exceedingly rare among individuals sampled in 2004 (data not shown) and 2005 (Fig. 1). The few fish that died after being sampled but before being euthanized were among the most heavily parasitized individuals (data not shown), suggesting that high infection intensities may cause mortality. As further evidence of morbidity, the fins of some highly infected individuals appeared frayed. Frayed fins are a common sign of heavy infection intensity for *G. eos* infection among other host species (Lyles 1990). More in depth study of the pathogenicity of *G. eos* is required.

There are a number of processes other than those related to parasites that could provide an advantage to sex in this system. These processes could be very important and could overwhelm or discount any advantage provided by resistance to parasites. Alternatively, these non-parasite-related processes could complement the role of parasites or they could be inconsequential. The dependence by gynogenetic asexual *Phoxinus* on sperm from the sexual species for reproduction is, however, likely a factor contributing to the maintenance of the sexual species. One would think that male *Phoxinus* should evolve a preference for conspecific sexual females in order not to waste energy and sperm (and hence loose fitness) by mating with asexuals. Moore and McKay (1971) and Moore (1975) have modeled the expected maximum number of asexuals that could coexist with their sexual relatives if males were selective in who they mated with, preferring not to waste their sperm on the asexual females. Their models were based on the hybridogenic *Poeciliopsis* Regan, 1913 (Pisces: Poeciliidae) system (Vrijenhoek and Schultz 1974). It is not known whether male *P. eos* practice assortative mating. Determining the degree of preference by male *Phoxinus* for mating with conspecific sexual females is an important next step in determining how sex is maintained in this system. Even if there is some preference for mating

Fig. 2. Median intensities of *G. eos* infection in samples of sexually and asexually reproducing *Phoxinus* from Algonquin Park. Species pairs within samples taken from the same lake are connected with a solid line. Infection intensities in A include males and females, while infection intensities in B are for female *Phoxinus* only. In both cases (A and B), there were significantly more parasites on asexual than sexual *Phoxinus* (see text for statistical analysis).



with conspecifics, one might expect that the asexual *Phoxinus*, given their twofold advantage in reproductive rate, could drive themselves and their sexual relative to extinction by rising in frequency until the hybrid females dilute the pool of sexual females to the point where all males are mating with hybrid females. Hence, if sexual *Phoxinus* benefit from increased resistance to parasitism, then the Red Queen may play a role along with any assortative mating in maintaining sex in the *Phoxinus* species complex.

Depending on who is counting, there are around 20 different plausible hypotheses for how sex evolved and how it is maintained when faced with being replaced by asexual reproduction (Kondrashov 1993). Either at least 95% of the theorists who came up with these theories were wrong, or there is some room for pluralism in the explanation of the maintenance of sex. Researchers have explicitly modeled how more than one hypothesis can contribute simultaneously to an advantage of sex (Howard and Lively 1994, 1998; West et al. 1999), and supporting empirical evidence has been provided (Buckling et al. 2006). It has also been noted that the conditions required for any hypothesis to act alone are often extreme and unreasonable. Otto and Nuismer (2004) have shown that parasites should only provide an advantage to sex given a relatively narrow range of parameter values and a small subset of ecological and genetic interactions. Mutation accumulation hypotheses (Muller 1964; Kondrashov 1982, 1988), similarly, require extremely high mutation rates to provide an advantage to sex (West et al. 1999). A more reasonable mutation rate might provide a smaller advantage to sex that, when combined with an advantage to sex owing to parasite resistance, may be enough to balance the substantial cost of sex. If multiple processes can act together to provide enough of an advantage to balance the cost of sex, then the conditions required for any particular process to be important can be relaxed. Showing that *G. eos* does infect asexual *Phoxinus* more than sexual *Phoxinus* therefore lends support to a role for parasites in the maintenance of sex in the *Phoxinus* species complex even if *G. eos* are not extremely virulent.

There have been a number of explicit comparisons of parasite loads on related sexual and asexual species. Convincing support for the Red Queen hypothesis has been provided by a continuing study of a parthenogenic snail species in New Zealand (Lively 1987, 1989, 1999; Jokela and Lively 1995; Jokela et al. 1997, 2003; Lively and Dybdahl 2000), as well as by studies of parasitism in *Poeciliopsis* fish (Lively et al. 1990), lizards (Moritz et al. 1991), flatworms (Michiels et al. 2001), and plants (Busch et al. 2004). A number of studies, however, have found evidence contradictory to the Red Queen (Brown et al. 1995; Hanley et al. 1995; Weeks 1996; Tobler et al. 2005). Although a prediction from the Red Queen hypothesis initially motivated the present study, the result could, in fact, be explained by a variety of hypothetical mechanisms. For example, the asexual *Phoxinus* species arose via hybridization, and a number of studies have indicated that hybrids can be more susceptible to parasitism than their parental species (Sage et al. 1986; Dupont and Crivelli 1988; Mason and Clark 1990; Rhymer et al. 1994). Alternatively, the accumulation of deleterious mutations in the asexual species (Muller 1964; Kondrashov 1982, 1988) could result in individuals with poorer condition having generally degraded immune systems and greater susceptibility to parasitism (see Hakoyama et al. 2001). Other studies have shown that asexual species derived from sexual species can suffer from frequent developmental abnormalities (Lamb and Willey 1979; Wetherington et al. 1987; Corley and Moore 1999), which could result in degraded immune systems and greater susceptibility to parasitism. The asexual *Phoxinus* species could also differ ecologically in such a way as to influence its tendency to be parasitized by *G. eos*. Previous studies have suggested that asexual *Phoxinus* have a broader ecological niche than their sexual progenitors and that the asexuals may use marginal habitat as a refuge from competition with the sexuals (Schlosser et al. 1998). Whether or not any ecological difference significantly affects the relative susceptibility to parasitism of asexual and sexual *Phoxinus* is unknown.

Acknowledgements

We are grateful to Aneil Agrawal, Don Jackson, and Brian Shuter for helpful discussion and comments. We owe thanks to Katherine Goddard, Isaak Schlosser, and David Cone for useful advice. All the fieldwork was based out of the Harkness Laboratories for Fisheries Research in Algonquin Park and we owe gratitude to Ontario Parks and the Ontario Ministry of Natural Resources for allowing this project to proceed. We also thank two anonymous reviewers for their helpful comments on an earlier draft of the manuscript. This project was funded by the Natural Sciences and Engineering Research Council of Canada and the Department of Zoology at the University of Toronto.

References

Agrawal, A.F. 2001. Sexual selection and the maintenance of sexual reproduction. Nature (London), **411**: 692–695. doi:10.1038/35079590. PMID: 11395771.

Barash, D.P. 1976. What does sex really cost? Am. Nat. **110**: 894–897.

Barton, N.H. 1995. A general model for the evolution of recombination. Genet. Res. **65**: 123–144. PMID: 7605514.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Croom Helm Ltd., London.

Brown, S.G., Kwan, S., and Shero, S. 1995. The parasitic theory of sexual reproduction: parasitism in unisexual and bisexual geckos. Proc. R. Soc. Lond. B Biol. Sci. **260**: 317–320.

Buckling, A., Wei, Y., Massey, R.C., Brockhurst, M.A., and Hochberg, M.E. 2006. Antagonistic coevolution with parasites increases the cost of host deleterious mutations. Proc. R. Soc. Lond. B Biol. Sci. **273**: 45–49.

Busch, J.W., Neiman, M., and Koslow, J.M. 2004. Evidence for maintenance of sex by pathogens in plants. Evolution, **58**: 2584–2590. PMID: 15612300.

Charlesworth, B. 1980. The cost of sex in relation to mating system. J. Theor. Biol. **84**: 655–671. PMID: 7431946.

Charlesworth, B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. Genet. Res. **55**: 199–221. PMID: 2394378.

Cone, D.K., and Odense, P.H. 1984. Pathology of five species of *Gyrodactylus* Nordmann, 1832 (Monogenea). Can. J. Zool. **266**: 471–476.

Corley, L.S., and Moore, A.J. 1999. Fitness of alternative modes of reproduction: developmental constraints and the evolutionary maintenance of sex. Proc. R. Soc. Lond. B Biol. Sci. **266**: 471–476.

Crow, J.F., and Kimura, M. 1965. Evolution in sexual and asexual populations. Am. Nat. **99**: 439–450. doi:10.1086/282389.

Darwin, C.R. 1862. On the two forms, or dimorphic condition, in the species *Primula*, and on their remarkable sexual relations. J. Proc. Linn. Soc. (Bot.), **6**: 77–96.

Doeringsfeld, M.R., Schlosser, I.J., Elder, J.F., and Evenson, D.P. 2004. Phenotypic consequences of genetic variation in a gynogenetic complex of *Phoxinus eos–neogaeus* clonal fish (Pisces: Cyprinidae) inhabiting a heterogeneous environment. Evolution, **58**: 1261–1273. PMID: 15266975.

Dupont, F., and Crivelli, A.J. 1988. Do parasites confer a disadvantage to hybrids? A case study of *Alburnus alburnus* × *Rutilus rubilio*, a natural hybrid of Lake Mikri Prespa, Northern Greece. Oecologia (Berl.), **75**: 587–592. doi:10.1007/BF00776424.

Elder, J.F., and Schlosser, I.J. 1995. Extreme clonal uniformity of *Phoxinus eos/neogaeus* gynogens (Pisces: Cyprinidae) among variable habitats in northern Minnesota beaver ponds. Proc. Natl. Acad. Sci. U.S.A. **92**: 5001–5005. PMID: 7761438.

Fisher, R.A. 1930. The genetical theory of natural selection. Claredon Press, Oxford.

Goddard, K.A., Dawley, R.M., and Dowling, T.E. 1989. Origin and genetic relationships of diploid, triploid, and diploid–triploid mosaic biotypes in the *Phoxinus eos–neogaeus* unisexual complex. *In* Evolution and ecology of unisexual vertebrates. *Edited by* R.M. Dawley and J.P. Bogart. New York State Education Department, Albany. pp. 269–280.

Goddard, K.A., Megwinoff, O., Wessner, L.L., and Giaimo, F. 1998. Confirmation of gynogenesis in *Phoxinus eos–neogaeus* (Pisces: Cyprinidae). J. Hered. **89**: 151–157. doi:10.1093/jhered/89.2.151.

Hakoyama, H., Nishimura, T., Matsubara, N., and Iguchi, K. 2001. Difference in parasite load and nonspecific immune reaction between sexual and gynogenetic forms of *Carassius auratus*. Biol. J. Linn. Soc. **72**: 401–407. doi:10.1006/bijl.2000.0507.

Hamilton, W.D. 1980. Sex versus non-sex versus parasite. Oikos, **35**: 282–290.

Hamilton, W.D. 1990. Memes of Haldane and Jayakar in a theory of sex. J. Genet. **69**: 17–32.

Hanley, K.A., Fisher, R.N., and Case, T.J. 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. Evolution, **49**: 418–426. doi:10.2307/2410266.

Hastings, I.M. 1999. The costs of sex due to deleterious intracellular parasites. J. Evol. Biol. **12**: 177–183. doi:10.1046/j.1420-9101.1999.00019.x.

He, X., and Lodge, D.M. 1990. Using minnow traps to estimate fish population size: the importance of spatial distribution and relative species abundance. Hydrobiologia, **190**: 9–14. doi:10.1007/BF00020683.

Howard, R.S., and Lively, C.M. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature (London), **367**: 554–557. doi:10.1038/367554a0. PMID: 8107824.

Howard, R.S., and Lively, C.M. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. Evolution, **52**: 604–610. doi:10.2307/2411094.

Jaenike, J. 1978. An hypothesis to account for the maintenance of sex within populations. Evol. Theory, **3**: 191–194.

Jokela, J., and Lively, C.M. 1995. Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution, **49**: 1268–1271. doi:10.2307/2410451.

Jokela, J., Lively, C.M., Dybdahl, M.F., and Fox, J.A. 1997. Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. Ecology, **78**: 452–460.

Jokela, J., Lively, C.M., Dybdahl, M.F., and Fox, J.A. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biol. J. Linn. Soc. **79**: 165–181. doi:10.1046/j.1095-8312.2003.00181.x.

Kondrashov, A.S. 1982. Selection against harmful mutations in large sexual and asexual populations. Genet. Res. **40**: 325–332. PMID: 7160619.

Kondrashov, A.S. 1988. Deleterious mutations and the evolution of sexual reproduction. Nature (London), **336**: 435–440. doi:10.1038/336435a0. PMID: 3057385.

Kondrashov, A.S. 1993. Classification of hypotheses on the advantage of amphimixis. J. Hered. **84**: 372–387. PMID: 8409359.

Lamb, R.Y., and Willey, R.B. 1979. Are parthenogenetic and related bisexual insects equal in fertility? Evolution, **33**: 774–775. doi:10.2307/2407801.

Lively, C.M. 1987. Evidence from a New-Zealand snail for the maintenance of sex by parasitism. Nature (London), **328**: 519–521. doi:10.1038/328519a0.

Lively, C.M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. Evolution, **43**: 1663–1671. doi:10.2307/2409382.

Lively, C.M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. Am. Nat. **153**: S34–S47. doi:10.1086/303210.

Lively, C.M., Craddock, C., and Vrijenhoek, R.C. 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature (London), **344**: 864–866. doi:10.1038/344864a0.

Lively, C.M., and Dybdahl, M.F. 2000. Parasite adaptation to locally common host genotypes. Nature (London), **405**: 679–681. doi:10.1038/35015069. PMID: 10864323.

Lyles, A.M. 1990. Genetic variation and susceptibility to parasites: *Poecilia reticulata* infected with *Gyrodactylus turnbulli*. Ph.D. thesis, Department of Ecology, Evolution and Behavior, Princeton University, Princeton, N.J.

Mason, J.R., and Clark, L. 1990. Sarcosporidiosis observed more frequently in hybrids of mallards and American black ducks. Wilson Bull. No. 102. pp. 160–162.

May, R.M., and Anderson, R.M. 1983. epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Lond. B Biol. Sci. **219**: 281–313. PMID: 6139816.

Maynard Smith, J. 1976. A short-term advantage for sex and recombination through sib-competition. J. Theor. Biol. **63**: 245–258. PMID: 1011844.

Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge.

Michiels, N.K., Beukeboom, L.W., Pongratz, N., and Zeitlinger, J. 2001. Parthenogenetic flatworms have more symbionts than their coexisting, sexual conspecifics, but does this support the Red Queen? J. Evol. Biol. **14**: 110–119. doi:10.1046/j.1420-9101.2001.00249.x.

Moore, W.S. 1975. stability of small unisexual–bisexual populations of *Poeciliopsis* (Pisces: Poeciliidae). Ecology, **56**: 791–808.

Moore, W.S., and McKay, F.E. 1971. Coexistence in unisexual species complexes of *Poeciliopsis* (Pisces: Poeciliidae). Ecology, **52**: 791–799. doi:10.2307/1936026.

Moritz, C., McCallum, H., Donnellan, S., and Roberts, J.D. 1991. Parasite loads in parthenogenetic and sexual lizards (*Heteronotia binoei*): support for the Red Queen Hypothesis. Proc. R. Soc. Lond. B Biol. Sci. **244**: 145–149.

Muller, H.J. 1964. The relation of recombination to mutational advance. Mutat. Res. **1**: 2–9.

Neuhauser, M., and Poulin, R. 2004. Comparing parasite numbers between samples of hosts. J. Parasitol. **90**: 689–691. PMID: 15357054.

New, J.G. 1962. Hybridization between two cyprinids, *Chrosomus eos* and *Chrosomus neogaeus.* Copeia, 1962: 147–152.

Otto, S.P., and Barton, N.H. 2001. Selection for recombination in small populations. Evolution, **55**: 1921–1931. PMID: 11761054.

Otto, S.P., and Nuismer, S.L. 2004. Species interactions and the evolution of sex. Science (Washington, D.C.), **304**: 1018–1020. doi:10.1126/science.1094072. PMID: 15143283.

Rhymer, J.M., Williams, M.J., and Braun, M.J. 1994. Mitochondrial analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*A. superciliosa*). Auk, **111**: 970–978.

Roughgarden, J. 1991. The evolution of sex. Am. Nat. **138**: 934–953. doi:10.1086/285261.

Sage, R.D., Heyneman, D., Lim, K.C., and Wilson, A.C. 1986. Wormy mice in a hybrid zone. Nature (London), **324**: 60–63. doi:10.1038/324060a0. PMID: 12356091.

SAS Institute Inc. 2000. JMP®. Version 4.0.2 [computer program]. SAS Institute Inc., Cary, N.C.

Schlosser, I.J., Doeringsfeld, M.R., Elder, J.F., and Arzayus, L.F. 1998. Niche relationships of clonal and sexual fish in a heterogeneous landscape. Ecology, **79**: 953–968. doi:10.2307/176592.

Siller, S. 2001. Sexual selection and the maintenance of sex. Nature (London), **411**: 689–692. doi:10.1038/35079578. PMID: 11395770.

Tobler, M., Wahli, T., and Schlupp, I. 2005. Comparison of parasite communities in native and introduced populations of sexual and asexual mollies of the genus *Poecilia.* J. Fish Biol. **67**: 1072–1082. doi:10.1111/j.0022-1112.2005.00810.x.

Vanvalen, L. 1973. A new evolutionary law. Evol. Theory, **1**: 1–30.

Vrijenhoek, R.C., Angus, R.A., and Schultz, R.J. 1978. Variation and clonal structure in a unisexual fish. Am. Nat. **112**: 41–55. doi:10.1086/283251.

Vrijenhoek, R.C., and Schultz, R.J. 1974. Evolution of a trihybrid unisexual fish (*Poeciliopsis*, Poeciliidae). Evolution, **28**: 306–319. doi:10.2307/2407332.

Weeks, S.C. 1996. A reevaluation of the Red Queen model for the maintenance of sex in a clonal-sexual fish complex (Poeciliidae: *Poeciliopsis*). Can. J. Fish. Aquat. Sci. **53**: 1157–1164. doi:10.1139/cjfas-53-5-1157.

West, S.A., Lively, C.M., and Read, A.F. 1999. A pluralist approach to sex and recombination. J. Evol. Biol. **12**: 1003–1012. doi:10.1046/j.1420-9101.1999.00119.x.

Wetherington, J.D., Kotora, K.E., and Vrijenhoek, R.C. 1987. A test of the spontaneous heterosis hypothesis for unisexual vertebrates. Evolution, **41**: 721–731. doi:10.2307/2408883.

Williams, G.C. 1975. Sex and evolution. Princeton University Press, Princeton, N.J.