Ecological differentiation between coexisting sexual and asexual strains of *Daphnia pulex*

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Summary

1. The widespread occurrence of sexual reproduction indicates that the benefits of sex can overcome its costs relative to asexual reproduction. Many closely related sexual and asexual taxa have different geographic distributions suggesting that their relative fitness may depend on the environment. However, support for such ecological differentiation is mainly based on correlative evidence, with experimental support being scarce, especially from the wild.

2. We studied ecological differentiation between asexual (obligate parthenogenetic) and sexual (cyclical parthenogenetic) *Daphnia pulex* in Southern Finland, where the two forms ('reproduction types') coexist regionally in a metapopulation. We found differences in several environmental factors between ponds inhabited by sexual and asexual populations (pH, calcium, oxygen, conductivity and absorbance).

3. A reciprocal competition experiment carried out directly in the natural ponds confirmed that sexuals and asexuals were ecologically differentiated; their relative fitness was found to depend on pH and calcium titres of the ponds.

4. Our study provides clear experimental evidence for the coexistence of closely related sexual and asexual taxa being mediated by ecological differentiation. In addition, the results of our competition experiment suggest that each of the reproduction types has a larger fundamental niche (absence of competition) than a realized niche (presence of competition). Thus, by temporarily creating competition-free space, metapopulation turnover may allow the reproduction types to occur in a wider range of habitats than under more stable population dynamics, and may thus further contribute to stabilize regional coexistence.

Key-words: Crustacean, geographic parthenogenesis, metapopulation, niche differentiation, paradox of sex

Introduction

The coexistence of closely related sexual and asexual strains is a fundamental paradox. Sexuals have immediate evolutionary costs relative to asexuals as they need to produce both males and females and pass only half of their genes to their offspring (Williams 1975; Maynard Smith 1978). Nonetheless, sexual reproduction is far more abundant than asexual reproduction indicating that sexuals can overcome these costs (Barton & Charlesworth 1998; Otto & Lenormand 2002; Agrawal 2006).

The prevailing understanding in ecology is that the long-term coexistence of reproductively isolated entities is mediated by ecological differentiation (Armstrong & McGehee 1980; Levine & HilleRisLambers 2009). Thus, the coexistence

of closely related sexuals and asexuals would be even more surprising, if they were ecologically equivalent. Indeed, there is some evidence that closely related sexuals and asexuals are ecological differentiated (Christensen 1960; Bell 1982; Lynch 1984): they often have different geographic distributions (Suomalainen, Saura & Lokki 1987; Mogie 1992). For instance in geographical parthenogenesis (Vandel 1928) the distribution patterns of sexual and asexual strains often correlate with environmental gradients; asexuals inhabit more northern and marginal habitats than their sexual counterparts. In addition, ecological correlates of the relative abundance of closely related sexual and asexual taxa have also been found at the small-scale, for instance in dandelions (Verduijn, Dijk & Damme 2004), fish (Schenck & Vrijenhoek 1986), parasitic wasps (Amat *et al.* 2006), planthoppers (Booij & Guldemond 1984) and aphids (Vorburger, Lancaster & Sunnucks 2003; Halkett *et al.* 2006).

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However, correlations between environmental factors and the relative abundance of sexual and asexual strains do not provide unequivocal evidence of ecological differentiation because the relative abundance can, for instance, also be affected by different colonization routes coinciding with ecological gradients. Thus, in order to test for ecological differentiation, it is necessary to establish a link between environmental factors and fitness. Previous studies both in field and laboratory have shown the differences in life-history responses to environmental factors between closely related sexual and asexual strains, or asymmetric competition between the two reproduction types (Browne, Davis & Sallee 1988; Weeks 1995; Barata *et al.* 1996; Vrijenhoek & Pfeiler 1997; Kovel & Jong 1999; Negovetic *et al.* 2001; Verduijn, Dijk & Damme 2004; Plenet *et al.* 2005; Agh *et al.* 2007; Wolinska & Lively 2008). These studies suggest that closely related sexuals and asexuals are often ecologically differentiated. However, to show that this promotes coexistence of sexuals and asexuals within the same region, it is necessary to show that the relative fitness between the two is inversed depending on ecological conditions, that is, that sexuals outperform asexuals under some field conditions and asexuals outperform sexuals under other field conditions. Yet such studies are still scant, especially experimental studies carried out in the wild. Overlapping zones, in which the distributions of sexuals and asexuals meet, provide suitable areas for such studies.

In Southern Finland, sexual (cyclical parthenogenetic) and asexual (obligate parthenogenetic) strains of the planktonic cladoceran *Daphnia pulex* regionally coexists in metapopulations consisting of local populations that inhabit freshwater rock pools on islands of the Baltic Sea archipelago (Ranta 1979; Hanski & Ranta 1983; Ward *et al.* 1994). These ponds vary in size and various, physiologically important environmental factors (Lagerspetz 1955; Potts & Parry 1964; Waervagen, Rukke & Hessen 2002; Glover & Wood 2005a,b; Pajunen & Pajunen 2007). Sexuals and asexuals often inhabit different ponds, but in a few instances, they co-occur in the same pond. It is yet unknown if this occurs less frequently than expected by chance, as would be predicted if there was strong ecological segregation.

In this study, we tested for ecological differentiation between the reproduction types by first assessing their relative distribution across the metapopulation system and ecological correlates of their occurrence, and second by assessing the effect of environmental factors on their relative fitness. The latter was achieved by setting up a competition experiment, in which sexual and asexual strains competed against each other in enclosures placed into the natural ponds. The combination of correlative and experimental methods provides a powerful approach for assessing ecological differentiation between sexuals and asexuals, and to investigate whether ecological differentiation could explain the distribution and regional coexistence of the two reproduction types. In addition to this, our results suggest ways in which regional coexistence of ecologically similar taxa in metapopulations can be stabilized by turnover dynamics creating temporarily competition-free habitat.

Materials and methods

Daphnia pulex shows a breeding system polymorphism with strains reproducing either by cyclical or by obligate parthenogenesis (Lynch 1983). Cyclical parthenogens reproduce clonally during the summer, but diapause stages, which are needed to survive winters and periods of drought, are produced sexually. In obligate parthenogens, diapause stages are also produced clonally, therefore the entire life cycle is clonal. We therefore refer to cyclical parthenogens as 'sexuals', and to obligate parthenogens as 'asexuals'. In Europe, asexual strains of *D. pulex* show a more northerly distribution than sexual strains (Ward *et al.* 1994), a pattern known as 'geographic parthenogenesis' (Vandel 1928). However, in our study area in southern Finland, the ranges overlap (Ward *et al.* 1994). The asexual strains in our study area belong to the North American clade of *D. pulex*, whereas the sexual strains belong to the European clade (C. R. Haag, unpublished data). Asexual strains of the North American clade have recently (i.e. within the last century) colonized large parts of Africa and Southern Europe (Mergeay, Verschuren & De Meester 2006), but it is unknown when colonization of Southern Finland took place. Unlike in many other sexual–asexual systems, the asexuals in our study area are diploids, not polyploids (Ward *et al.* 1994). This excludes the possibility that differences between sexuals and asexuals are caused by ploidy levels.

In spring 2008, soon after hatching from diapause, we recorded the presence or absence of *D. pulex* from all ponds in which the species had previously been found ($N \approx 100$ ponds), in an area of about 6×6 km around Tvärminne Zoological Station (59°50′ N, 23°15′ E, Fig. 1). The central part of this area has been monitored for the presence and absence of *Daphnia* species since 1982 (Pajunen 1986; Pajunen & Pajunen 2003), so we are confident that we have found all *D. pulex* populations in this area, while it is possible that we have
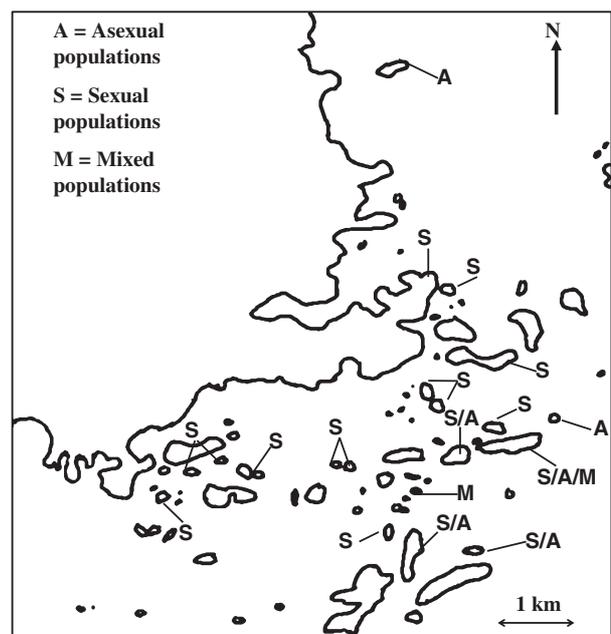

Fig. 1. Study area and distribution of *Daphnia pulex* populations. The filled circle indicates the location of Tvärminne Zoological Station.

missed some new colonisations in the surrounding area. For all 55 ponds (on 20 different islands) in which we found a *D. pulex* population, we determined the breeding system of the population by genotyping at least 11 individuals at the locus PGI (phosphoglucose isomerase, EC 5.3.1.9.) using cellulose acetate electrophoresis (Hebert & Beaton 1993). The study of Ward *et al.* (1994) found this locus to be diagnostic for differentiating sexuals vs. asexuals in our study area, with sexuals and asexuals being both homozygous for different alleles. We confirmed this by assessing segregation at other, heterozygous loci in hatchlings from diapause eggs having either 'sexual' or 'asexual' alleles at PGI (strains from 24 sexual and 12 asexual populations, C.R. Haag, unpublished data). While PGI appears to be a diagnostic marker for differentiating sexuals and asexuals in Southern Finland, it is unclear whether this would also be the case elsewhere.

To assess the environmental factors of the ponds, we measured the amount of dissolved oxygen (mg L^{-1}), pH, conductivity (μS cm^{-1}), calcium (mg L^{-1}), and absorbance (at 400 nm) of the water at five different dates between 10/5/2008 and 1/8/2008. At each date, we analysed only ponds containing a planktonic population of *D. pulex*; 55 ponds were analysed at least once (many ponds were temporarily dry in June and were thus not analysed) and 47 of them were analysed four or five times. All measurements were carried out in the laboratory using 100 mL water samples. Oxygen and pH were measured on the day of sampling, conductivity, calcium and absorbance on the day of sampling or the following day (storage at $+8$ °C). The most likely factor to change during transport and short-term storage is the oxygen content, which showed highly repeatable measurements between field and laboratory ($N = 19$, $R^2 = 0.857$, $P < 0.0001$). Dissolved oxygen was measured with a YSI oxygen-meter (model 95: YSI Inc., Yellow Springs, Ohio, USA), conductivity with a CDM-83 conductivity-meter (Radiometer, Copenhagen, Denmark, temperature coefficient $= 1.97\%/$°C, cell constant $= 0.980$ cm^{-1}, reference temperature $= 25$ °C), and pH with a Jenway 3510 pH-meter (Bibby Scientific Limited, Staffordshire, UK). Calcium concentration was measured with the Merck calcium titration kit (1.11110.0001; Merck KGaA, Darmstadt, Germany) and absorbance was measured with a Shimadzu UV-2501PC (Shimadzu, Kyoto, Japan) spectrophotometer at 400 nm after filtering the water through a 0.22-μm filter. We also recorded the volume of the ponds, though, due to unusually strong fluctuations in water level of the ponds cause by a period of draught, we only obtained reliable measurement for 26 ponds (17 inhabited by sexuals, nine by asexuals). Volume was estimated by assuming the shape of an inverted pyramid (maximal length × maximal perpendicular width × maximal depth/3). For all ponds, we classified them into large and small, roughly corresponding to whether their normal volume exceeded 100 L or not.

COMPETITION EXPERIMENT

The aim of the competition experiment was to record the changes in relative frequency of sexuals vs. asexuals, when individuals of the two reproduction types were placed at initially equal frequency into enclosures in natural ponds. The changes in relative frequency were used as a measure of relative performance, just as in competition experiments between different strains of bacteria or yeast (Hartl & Clark 1997). Such competition experiments carried out across several clonal generations (3–4 generations in our experiment), cover a large part of the *Daphnia* life cycle, including fecundity and survival, but they do not include diapause egg production and survival of diapause eggs. While this may be a shortcoming of this measure of performance, our experiment was carried out during a period of the season when both sexuals and asexuals reproduce mainly by clonal production of female offspring. This allowed us to estimate the relative performance while minimizing the potentially confounding influence of the presence of males and production of diapause eggs. Competition experiments resemble the condition in nature when both reproduction types co-occur within a single pond. These experiments cannot, however, determine whether changes in relative frequency are caused by competitive interactions or whether they are due to growth rate differences that would also occur in the absence of competition. Yet, under the assumption that the total number of individuals in a pond is limited and that the results of a competition experiment can be extrapolated over longer periods of time, they still estimate which of the two reproduction types gets to dominate the pond and at which speed this happens.

To test whether the relative performance of sexuals and asexuals depends on the environment, the competition experiment was carried out in ponds ('recipient ponds') that were either inhabited by sexuals or by asexuals ('current occupant') and differed in environmental factors. The null-hypothesis with this experimental setup is that the changes in relative frequency do not depend on the current occupant nor on the environmental factors of the recipient ponds. To test for ecological differentiation, we considered two hypotheses: Under the first, the changes in relative frequency depend on the current occupant. This would indicate ecological differentiation if the asexuals do relatively better in ponds inhabited by asexuals and sexuals in ponds inhabited by sexuals, and would also indicate that sexuals and asexuals in the recipient ponds occur in environments corresponding to their ecological preferences (note that this outcome would suggest ecological differentiation independent on whether there would also be a significant effect of the measured environmental factors on the relative performance of sexuals and asexuals). Under the second hypothesis, the relative performance of sexuals and asexuals would depend on the environmental factors of the pond, but not on the current occupant. This would also indicate ecological differentiation, but it would also suggest that the current distribution of sexuals and asexuals does not correspond to their optimum habitat, for instance because in the absence of competition, they may sometimes colonize suboptimal habitats.

Animals from 12 sexual and 10 asexual populations were used in the competition experiment. These were randomly assigned to 12 sexual–asexual competitor pairs, two asexual populations being used twice (the results did not change qualitatively, if these data were pooled). To equalize conditions for all animals before the onset of the experiment, samples from each population were grown outside for 4 weeks without adding food in 7.5 L of 48 μm-filtered water from a rock pool containing no *Daphnia*. To avoid sampling of clonal copies from sexual populations, we sampled all ponds shortly after hatching from diapause. The number of individuals sampled varied among populations (mean $\pm$ SD $= 112 \pm 62.2$ for sexual populations). We tried to obtain 150 individuals per population but in some populations not enough individuals were available in early spring. In asexual populations, the number of individuals sampled was lower (mean $\pm$ SD $= 33 \pm 30.1$), but at any rate, they are expected to be mostly clonal copies [an earlier study of *D. pulex* in North-America has found an average of 1.7 asexual clones per population (Wilson & Hebert 1992)]. Note also that the paired design of our experiment (replicates of the same competitor pair were tested in different ponds, and tests were carried out within pairs, see below) controls for potentially confounding differences between pairs (such as the different sample sizes used to establish the pre-experimental populations). Due to an exceptional drought in early summer 2008, no samples could be obtained from five asexual populations. Instead, we used samples from the same ponds that had been obtained in the previous

year and had since been cultured in 80 L buckets outdoors. There was no difference in the competition results between pairs involving asexuals sampled in 2007 and pairs involving asexuals sampled in 2008 (one-way ANOVA: $F_{1,11} = 0.056$; $P = 0.82$, year of sampling as explanatory variable and w_{asex} (see below) averaged for each pair as response variable).

Six ponds inhabited by sexuals and six ponds inhabited by asexuals were used as recipient ponds for the competition enclosures. Each enclosure consisted of a 1.5-L wide-necked bottle with a hole of 58 mm diameter covered with 150 μm filter net which prevented *Daphnia* from moving between the enclosure and the pond, but allowed pond water to move freely. In a few cases, in which the filter nets became clogged by excessive algae growth during the experiment, the algae were removed. Each competitor pair was placed into one enclosure in each of four recipient ponds, two of which were inhabited by sexuals and two by asexuals. Thus, each of the twelve recipient pond had four enclosures (total N enclosures = 48). To avoid potential confounding effects of local adaptation to a specific pond, the animals of both strains used in the competition always originated from a different pond than the one in which competition was carried out.

To set up the experiment, on 2/3 July 2008 we transferred 50 asexual and 50 sexual adult females (which were free of known parasites) into each enclosure. We attempted to equalize the number of brood-carrying females and the body size distribution in all replicates, but where this was not possible, equalization was done at least among the four replicates of the same pair (analyses were carried out within pairs, see below). We then placed the enclosures into the recipient ponds, by letting them float freely just below the water level (Fig. 2). One month later (30 July–3 August 2008), the enclosures were taken to the laboratory, where we counted the number of individuals and estimated the frequencies of the sexuals and asexuals by genotyping 47 individuals from each enclosure at PGI.

STATISTICAL ANALYSES

Parallel gradients in distribution of the reproduction types and environmental factors could lead to situations resembling that expected under ecological differentiation (see Introduction). We assessed the presence of such gradients by carrying out a spatial autocorrelation analysis using SAM v3·0 (Rangel, Diniz-Filho & Bini 2006). JMP 7·0·2 (SAS Institute 2007) was used for all other statistical analyses. Tests for differences in environmental factors between the ponds inhabited by sexuals and asexuals were carried out with logistic regression (the presence of sexual vs. asexuals being the response variable and the different environmental factors being the explanatory variables), using mean values of environmental factors for each pond (averaged across sampling events). Values of conductivity, absorbance, calcium and volume were ln-transformed prior to analysis to approximate a normal distribution. To reduce the amount of different explanatory variables, we also conducted a principal component analysis, though the volume was not included in this analysis, because we had values only for about half of the ponds.

In the competition experiment, the fitness of asexuals relative to sexuals (w_{asex}) was estimated according to the change in their relative frequencies: $w_{asex} = \mathrm{Ln}\ (f_{asex}/(1 - f_{asex}))$, where f_{asex} is the frequency of asexuals (Hartl & Clark 1997). Note also that $w_{sex} = -w_{asex}$. For samples containing only sexual or only asexual individuals ($N = 18$ out of 44 populations), this fitness estimate could not be calculated. Instead, we used a slightly conservative estimate by assuming that one individual (instead of zero) was of the minority strain. All analyses accounted for the pairwise design of the study: we
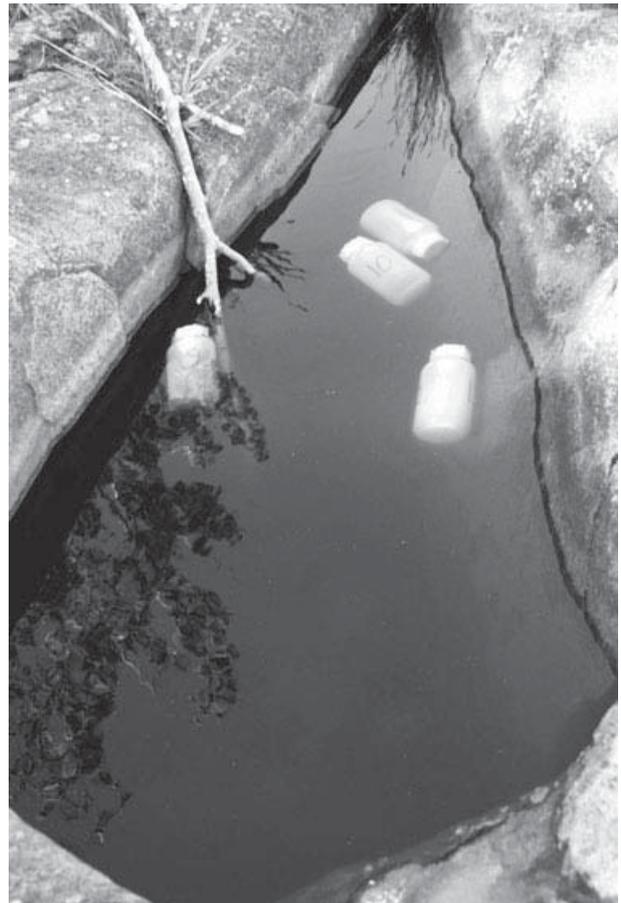
Fig. 2. Example of experimental pond containing competition enclosures.

first tested whether within pairs, asexuals did relatively better in replicates that were placed into ponds occupied by asexuals and sexuals did relatively better in replicates placed into ponds inhabited by sexuals. This test of an effect of the current occupant evaluates the first hypothesis (see above). Second, we tested whether within pairs, the relative performance of sexuals and asexuals varied among replicates depending on the environmental factors of the recipient ponds. This evaluates the second hypothesis (see above). The first test was carried out by testing for an effect of the current occupant of the pond (sexual or asexual) on w_{asex} in a mixed model (using REML), with w_{asex} as response variable, 'current occupant' as fixed factor, and pair (alone and as interaction with 'current occupant') and pond (nested within 'current occupant') as random variables ($w_{asex} \sim$ current_occupant, pair(random), current_occupant × pair(random), pond[current_occupant](random)). This model tests for an effect of the current occupant on w_{asex} while accounting for the factors pair and pond, thus adequately accounting for our data structure with replicates of the same pairs tested in different recipient ponds, and with each recipient pond having four enclosures with different pairs. Note that by accounting for the factor pond in this test, we implicitly account for differences in environmental factors: if environmental factors have a strong influence on w_{asex}, we would expect the factor pond to explain a large proportion of the variance in the REML model. The interaction (current_occupant × pair) was removed from the final model because it was non-significant, explained almost no variance, and was not a planned comparison; however, this had no qualitative effect on the results.

To test our second hypothesis (i.e. to test explicitly for an effect of environmental factors on w_{asex} within pairs, without accounting for the current occupant), we repeated the above model without 'current occupant' ($w_{asex} \sim$ pair(random), pond(random)). We then analysed weighted contrasts between ponds with weights given to ponds according to the ranked values of their environmental factors (each factor was tested in a separate model). Thus, for each environmental factor, we ranked the ponds according to their values for this factor, and the six ponds with the lower values obtained negative weights (the highest negative weight was given to the pond with the lowest value), whereas the six ponds with the higher values obtained positive weights (the highest positive weight was given to the pond with the highest value). The actual weights were −0·2857, −0·2381, −0·1905, −0·1429, −0·0952, −0·0476, 0·0476, 0·0952, 0·1429, 0·1905, 0·2381 and 0·2857. These add up to −1 for the negative values and to 1 for the positive values and subsequent ranks have equal differences in weight. This analysis comes down to testing whether, within pairs, w_{asex} varies among ponds according to the rank values of the ponds for environmental factors.

Results

Fourteen ponds were inhabited by asexuals, 39 by sexuals, and two by both sexuals and asexuals. Because of their low number, the two mixed populations were excluded from further analysis. The distribution of sexuals and asexual shows that the latter tended to occur mostly in the east of the study area, whereas sexuals occurred throughout (Fig. 1). The distribution of sexual vs. asexual populations as well as the environmental factors showed a positive spatial autocorrelation at short distances (Table 1), indicating that the ponds close to each other were more similar than expected by chance. However, we did not detect any clear gradient (i.e. linearly decreasing autocorrelation with distance), with significantly negative values at the extreme distances being mainly caused by one isolated island in the north-east, which was only inhabited by asexuals and which differed strongly in most environmental factors from ponds on most other islands. Thus, the spatial autocorrelation analysis did not provide clear evidence for parallel gradients in distribution of the reproduction types and environmental factors.

Single environmental factors and principal components differed clearly and significantly between ponds inhabited by sexuals and asexuals (Fig. 3a, Table 2). Even though the values of ponds inhabited by sexuals and asexuals overlapped, asexuals tended to occur in ponds with a higher conductivity, pH, oxygen content, calcium concentration and lower absorbance than sexuals. The differences, except for oxygen, were
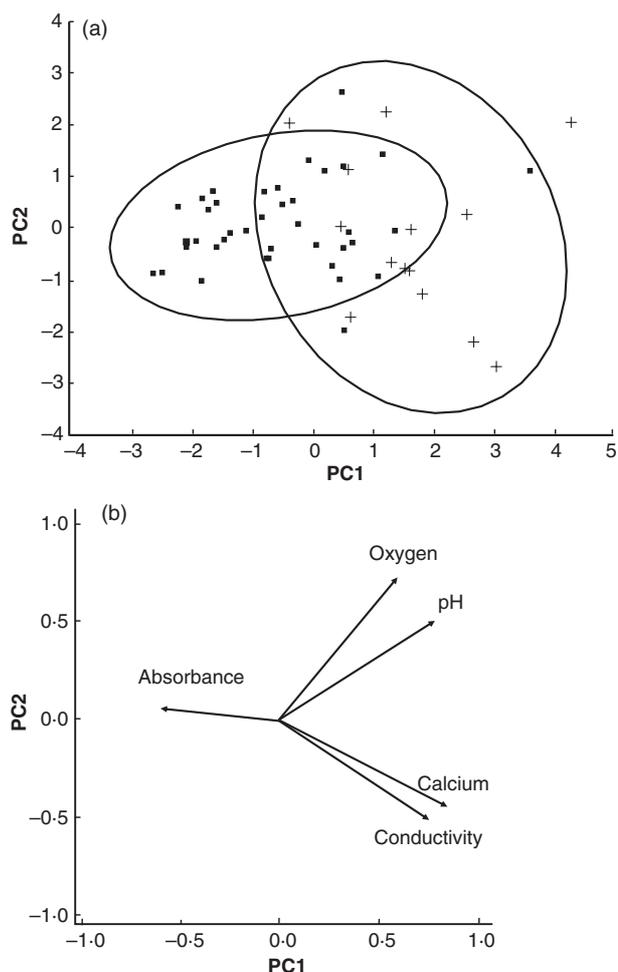

Fig. 3. (a) Principal component analysis of environmental factors with 90% density ellipses. Asexual ponds indicated with + and sexual ponds with ■. The first component explains 50·9% of the variation and the second component 23·4%. (b) Loadings of environmental factors.

also significant if islands instead of ponds were considered as units of replication (logistic regression with presence of sexuals/asexuals as response variable and mean values for all ponds inhabited by sexuals and for all ponds inhabited by asexuals on each island as explanatory variable). In addition, asexuals tended to occur in smaller ponds than sexuals (logistic regression using the 26 ponds on which we had data (Table 2) and Fisher's exact test on size categories of all ponds, $P = 0·027$).

Table 1. Moran's I/I_{max} values for current occupant, environmental factors and first principal component (PC1)

Distance (m)	Count	Current occupant	Conductivity	pH	Oxygen	Calcium	Absorbance	PC1
0–25	25	**0·601**	**0·257**	**0·253**	0·223	**0·272**	**0·466**	**0·548**
25–200	51	0·241	−0·146	0·04	0·032	−0·17	−0·124	−0·229
200–1000	217	−0·14	−0·252	0·146	0·016	−0·242	0·103	−0·017
1000–2000	346	**0·305**	−0·035	−0·1	−0·185	0·175	−0·116	0·173
2000–6000	636	**−0·612**	−0·166	**−0·485**	−0·262	−0·346	−0·318	**−0·541**

Bold values are represented as $P < 0·05$.

5

Table 2. Environmental factors of ponds inhabited by sexuals and asexuals

	N_{sex}, N_{asex}	$\chi^2_{d.f.} = 1$	Sex (mean $\pm$ SD)	Asex (mean $\pm$ SD)
Conductivity ($\mu S\ cm^{-1}$)	39, 14	14·01***	290·8 $\pm$ 252·4	973·9 $\pm$ 928·9
pH	39, 14	9·90**	6·81 $\pm$ 0·81	7·65 $\pm$ 0·81
Oxygen (mg L^{-1})	39, 14	3·86*	7·77 $\pm$ 1·10	8·51 $\pm$ 1·38
Calcium (mg L^{-1})	39, 14	14·21***	6·09 $\pm$ 4·82	23·63 $\pm$ 27·85
Absorbance	39, 14	12·77***	0·956 $\pm$ 0·539	0·495 $\pm$ 0·222
Volume (L)	17, 9	8·56**	529·9 $\pm$ 620·8	110·8 $\pm$ 105·1
First principal component	39, 14	23·13****	−0·578 $\pm$ 1·301	1·610 $\pm$ 1·202

Differences in means were tested by logistic regression. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
N_{sex} and N_{asex} indicate the number of ponds inhabited by either of the two reproduction types used in the analysis.

Table 3. Correlations between environmental factors

	1	2	3	4	5
1 Log absorbance					
2 Log calcium	−0·59*				
3 Oxygen	−0·42	0·26			
4 Log conductivity	−0·44	0·78***	0·13		
5 pH	−0·49	0·41**	0·48***	0·32	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ with Bonferroni corrections for multiple testing.

There were strong correlations between most environmental factors (Table 3), suggesting that if sexuals and asexuals are ecologically differentiated, they may be differentiated only with respect to a subset of factors, with the other factors being correlated markers of a certain type of environment. This was also suggested by the principal component analysis, where ponds inhabited by sexuals and asexuals differed along the first, but not the second component (Fig. 3a). The loadings of the environmental factors in the PCA are presented in Fig. 3b.

In the competition experiment, two replicates failed for technical reasons, and two because the populations in the enclosures became extinct; thus 11 instead of 12 pairs were used in the analyses. The final population sizes in non-extinct replicates varied from 50 to > 1500. Contrary to the hypothesis that asexuals would perform better in ponds inhabited by asexuals and sexuals in ponds inhabited by sexuals (our first hypothesis), the average relative fitness of asexuals was only slightly, and non-significantly, higher in ponds inhabited by asexuals than in ponds inhabited by sexuals (Table 4). The factor 'pair' explained only 6% of the variance and was non-significant when the model was analysed with traditional expected mean squares (EMS) rather than REML (Table 4). In contrast, the factor 'pond' explained 62% of the total variance in relative fitness under the REML model and was highly significant in the EMS model (Table 4). This suggests that the fitness of asexuals relative to sexuals was mainly influenced by some characteristic of the pond, and indeed, the contrast analysis showed that a significant part of this variation could be explained by environmental factors (Table 4, Fig. 4).

Discussion

ECOLOGICAL DIFFERENTIATION BETWEEN SEXUALS AND ASEXUALS

The finding of clear environmental differences between ponds inhabited by sexuals and asexuals suggests ecological differentiation between the two reproduction types. However, with the observational data alone, we could not exclude the possibility that the environmental differences are explained by spatial segregation (e.g. due to different colonization routes of sexuals vs. asexuals) together with environmental gradients (e.g. pH and salinity being higher in the eastern part of the study area where most asexual populations were found). While the spatial autocorrelation analysis did not provide clear evidence for parallel gradients in distribution of the reproduction types and environmental factors, it does not definitely reject this hypothesis either. We therefore carried out a competition experiment directly in the natural ponds, and the results of this experiment provided clear evidence for ecological differentiation. As mentioned above, differences in habitat preferences and in responses to environmental factors between closely related sexual and asexual strains have been reported in several taxa. However, to our knowledge, this study is the first to provide a clear link between habitat differences and relative fitness in a form of a direct competition experiment in the field.

We found that sexual and asexual *D. pulex* strains from southern Finland tend to inhabit ponds that show opposite characteristics in relation to pH, oxygen, calcium, conductivity and absorbance even if only pH and calcium significantly affected their relative fitness in the experiment. These factors are likely to be influenced in opposite ways by the relative influx of sea water vs. humic acid-rich fresh water into the ponds. It is thus possible that asexuals typically inhabit ponds exposed to the sea, whereas sexuals typically occur in more sheltered locations with nearby vegetation. Yet rather than assessing the relative exposure to sea and vegetation, we decided to directly measure chemical parameters of the pond water; they are the likely reasons for ecological differentiation, given their strong physiological effects (Potts & Parry 1964; Glover & Wood 2005a,b). While our results do not allow us to conclusively identify which parameters cause

Table 4. Statistical tests of competition experiment

Testing the effect of the current occupant[a]

Explanatory variable	REML model				EMS model		
	F	d.f.	P	%	F	d.f.	P
Current occupant	0·25	1,10	0·63	n.a.	0·2	1,9·5	0·67
Pair	n.a.	n.a.	n.a.	6·3	1·43	10,20	0·24
Pond[Current occupant]	n.a.	n.a.	n.a.	61·6	6·71	10,20	0·0002
Parameter estimates for current occupant (unit w_{asex})							
	Estimate	SE					
Current occupant = sexuals	0·068	1·03					
Current occupant = asexuals	0·773	1·02					

Testing the effects of the environmental factors[a]

Explanatory variable	REML model				Contrasts among ponds[b]		
	F	d.f.	P	%	F	d.f.	P
Pair	n.a.	n.a.	n.a.	6·8			
Pond	n.a.	n.a.	n.a.	59·5			
pH					11·66	1,33·1	0·002
Calcium					23·1	1,35·1	<0·001
PC1					7·15	1,32·2	0·011
Conductivity					0·71	1,36·7	0·41
Oxygen					2·48	1,36·3	0·12
Absorbance					0·96	1,36·7	0·33

REML, restricted maximum likelihood; EMS, expected mean squares.
[a]The response variable for all analyses was w_{asex}.
[b]For this analysis, ponds were ranked according to the environmental factor tested and then a weighted contrast analysis was carried out with weights given to different ponds according to the rank order, as described in the text.
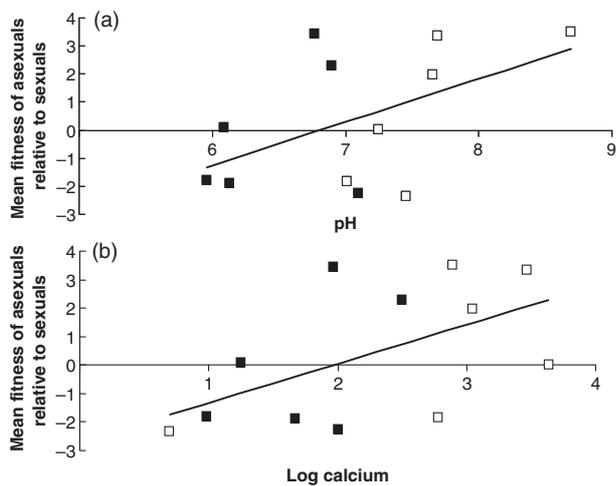

Fig. 4. Correlation between mean fitness of asexuals relative to sexuals and (a) pH and (b) ln transformed calcium. Filled squares indicate ponds inhabited by sexual, unfilled squares ponds inhabited by asexuals. Each point is an average of four replicates. The significance of the correlations was tested as described in the text.

ecological differentiation, previous studies on *Daphnia*, in accordance with our results, have shown an importance of pH, calcium, and salinity for physiology and ecological dif-ferentiation (Waervagen, Rukke & Hessen 2002; Glover & Wood 2005b). Also, the relative performance between different asexual *D. pulex* clones from Canada depends on the salinity, pH and temperature of their environments (Wilson & Hebert 1992; Weider, Frisch & Hebert 2010).

Despite the clear ecological differentiation, we found a large overlap in environmental factors between ponds occupied by sexuals and asexuals, and the outcome of the competition experiment was determined by environmental factors, but not by the current occupant. This suggests that the fundamental niches (i.e. the conditions that allow occurrence in the absence of competition) are larger than the realized niches in the presence of competition (Hutchinson 1957). It suggests that both reproduction types are absent from part of their ideal ponds, which may be explained by chance events due to the extinction and recolonization dynamics of the metapopulation (Pajunen & Pajunen 2003). This absence, in turn might allow the other reproduction type to temporarily occur in ponds where they have inferior competitive ability.

The occurrence of ecological differentiation does not exclude that other factors, such as genetic diversity, inbreeding levels of sexuals, and parasite load, also influence the relative fitness of sexuals and asexuals in this system (Haag & Ebert 2004; Tagg, Doncaster & Innes 2005; Haag *et al.* 2006). These and other unknown alternative factors may

indeed contribute to explain the distribution of the two breeding types in the metapopulation system. However, because these factors are expected to be constant among the different replicates of a pair (the level at which comparisons were made in our experiment), it seems unlikely that our finding of ecological differentiation is confounded by any substantial effect of these factors.

As mentioned above, our fitness estimate does not account for potential differences between sexuals and asexuals in the production of diapause eggs, survival of diapause eggs, as well as hatching and clonal growth just after hatching from diapause. It is possible that differences in some of these traits also contribute to explaining the patterns of occurrence and the relative performance of sexuals and asexuals in this system. However, our experiment has shown that the relative changes in frequencies, which form the basis of our fitness estimate, occur at a fast rate and may lead to the extinction of one of the competitors within only a fraction (about one-fifth) of the entire growing season. Once extinct or at low frequency, a reproduction type is expected to contribute less to the overall resting egg production than the more abundant reproduction type even if there were intrinsic differences in resting egg production between sexuals and asexuals. It is therefore likely that the changes in relative frequencies in our experiment provide a meaningful estimate of the relative performance of sexuals and asexuals in this system. However, independent of this assertion, our experiment shows ecological differentiation between sexuals and asexuals (at least with respect to their relative performance during clonal growth) for environmental factors that coincide with environmental differences between ponds inhabited by sexuals vs. asexuals.

COEXISTENCE OF SEXUALS AND ASEXUALS

Earlier studies have found correlative and also experimental evidence for ecological differences between closely related sexuals and asexuals also in other taxa (e.g. Schenck & Vrijenhoek 1986; Barata *et al*. 1996; Verduijn, Dijk & Damme 2004; Amat *et al*. 2006; Halkett *et al*. 2006). Together with these, our results suggest that ecological differentiation may be a key to coexistence of closely related sexuals and asexuals. Evolutionary models of the relative fitness of sexuals and asexuals often assume that sexuals and asexuals are ecologically equivalent. But if our results hold true for other sexual–asexual species pairs, it is questionable whether coexistence (even if only on a regional level) of ecologically equivalent sexuals and asexuals is common in nature. It is therefore perhaps not so surprising that in several instances where closely related sexuals and asexuals have been found to coexist, sexuals and asexuals belong to different lineages within a species, indicating that they started to diverge sufficiently long ago for ecological differentiation to evolve (e.g. Delmotte *et al*. 2002). In this respect, coexistence of closely related sexuals and asexuals may not differ much from the coexistence of closely related sexual species. However, one important difference is the presence of an intrinsic barrier to gene-flow (one species being asexual), which at least in theory might allow

coexistence of ecologically more similar taxa than with gene-flow.

REGIONAL COEXISTENCE IN METAPOPULATIONS

In many species, the fundamental niches are larger than the realized niches in the presence of ecologically similar (though not equivalent) species (Hutchinson 1957). Our study suggests that by temporarily creating competition-free patches, metapopulation turnover may thus promote the regional coexistence of ecologically similar taxa. This has also been suggested as a theory for the coexistence of three different *Daphnia* species (*D. pulex*, *Daphnia magna* and *Daphnia longisspina*) in a metacommunity in our study area (Hanski & Ranta 1983). This theory is based on asymmetric competition among species (one species being the unconditionally superior competitor) and a trade-off between competition and dispersal (i.e. the worst competitor being the best disperser). In contrast, we find that the competition between sexual and asexual *D. pulex* is not unconditionally asymmetrical, but rather that, due to ecological differentiation, each of the reproduction types has its 'safe havens', that is ponds, in which it is the superior competitor. Such safe havens, which may considerably stabilize the system, may also occur for competition among the three *Daphnia* species, which indeed have been found to slightly differ in their ecological preferences and distributions (Ranta 1979; Pajunen & Pajunen 2007).

GEOGRAPHICAL PARTHENOGENESIS

Many asexual taxa tend to be more northerly and marginally distributed than their close sexual relatives ('geographic parthenogenesis', Vandel 1928), including *D. pulex* in Europe (Ward *et al*. 1994). While our data do not show that any of the ecological differences we found between sexual and asexual *D. pulex* are related to environments that generally favour one breeding system (sexuals or asexuals) over the other, the observed patterns are reminiscent of geographic parthenogenesis. The asexuals tend to occur in ponds that are smaller and more exposed to the sea than sexuals, which occur in larger and more sheltered ponds. Unfortunately, we do not have long-term distribution data on sexuals and asexuals, but smaller and more exposed ponds may have higher extinction risks (e.g. due to waves sweeping the ponds during autumn storms). This is in line with several potential explanations for geographic parthenogenesis (Hörandl 2006), especially hypotheses that link geographic parthenogenesis to turnover rate (e.g. Gerritsen 1980; Lively, Craddock & Vrijenhoek 1990; Haag & Ebert 2004). In metapopulation systems, inbreeding due to genetic bottlenecks after colonization may be common in sexual populations (inbreeding does not occur in asexuals, because they do not mate). Because inbreeding is likely connected with colonization bottlenecks, it may be stronger in high-turnover populations (Haag *et al*. 2006), thus potentially explaining why sexuals preferentially occur in the more stable ponds.

It has recently been suggested that such small-scale geographic parthenogenesis exists also in other taxa (Verduijn, Dijk & Damme 2004) and that these differences found at the local or regional may translate into explaining geographic parthenogenesis at larger geographic scales. If ecological conditions preferred by asexuals predominate in the north and ecological conditions preferred by sexuals predominate in the south and if the reproduction types have spread to these geographic regions after they had become adapted to different environments, this might be a viable hypothesis also for the geographic parthenogenesis exhibited by *D. pulex* in Europe (Ward *et al.* 1994). Alternatively, ecological differentiation may be a consequence and not the reason of geographic parthenogenesis: sexuals and asexuals may have spread to southern Finland via different routes, sexuals from the south and asexuals from the north. During this spread, they may have adapted to the predominant ecological conditions in these areas, and the ecological differentiation they show currently in the overlap zone in southern Finland may thus be a consequence of the adaptation during this spread. While our data do not show which one (if any) of these hypotheses is true in *D. pulex*, our results do suggest that large-scale geographical parthenogenesis translates into small-scale ecological differentiation in an area where the two reproduction types coexist.

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References

Agh, N., Abatzopoulos, T.J., Kappas, I., Van Stappen, G., Rouhani, S.M.R. & Sorgeloos, P. (2007) Coexistence of sexual and parthenogenetic *Artemia* populations in Lake Urmia and neighbouring lagoons. *International Review of Hydrobiology*, **92**, 48–60.

Agrawal, A.F. (2006) Evolution of sex: why do organisms shuffle their genotypes. *Current Biology*, **16**, R696–R704.

Amat, I., Castelo, M., Desouhant, E. & Bernstein, C. (2006) The influence of temperature and host availability on the host exploitation strategies of sexual and asexual parasitic wasps of the same species. *Oecologia*, **148**, 153–161.

Armstrong, R.A. & McGehee, R. (1980) Competitive exclusion. *American Naturalist*, **115**, 151–170.

Barata, C., Hontoria, F., Amat, F. & Browne, R. (1996) Competition between sexual and parthenogenetic *Artemia*: temperature and strain effects. *Journal of Experimental Marine Biology and Ecology*, **196**, 313–328.

Barton, N.H. & Charlesworth, B. (1998) Why sex and recombination. *Science*, **281**, 1986–1990.

Bell, G. (1982) *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press, Berkeley, CA, USA.

Booij, C.J.H. & Guldemond, J.A. (1984) Distributional and ecological eifferentiation between asexual gynogenetic planthoppers and related sexual species of the genus *Muellerianella* (Homoptera, Delphacidae). *Evolution*, **38**, 163–175.

Browne, R.A., Davis, L.E. & Sallee, S.E. (1988) Effects of temperature and relative fitness of sexual and asexual brine shrimp *Artemia*. *Journal of Experimental Marine Biology and Ecology*, **124**, 1–20.

Christensen, B. (1960) A comparative cytological investigation of the reproductive cycle of an amphimictic diploid and a parthenogenetic triploid form of

Lumbricillus lineatus (OFM) (Oligochaeta, Enchytraeidae). *Chromosoma*, **11**, 365–379.

Delmotte, F., Leterme, N., Gauthier, J.P., Rispe, C. & Simon, J.C. (2002) Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. *Molecular Ecology*, **11**, 711–723.

Gerritsen, J. (1980) Sex and parthenogenesis in sparse populations. *American Naturalist*, **115**, 718–742.

Glover, C.N. & Wood, C.M. (2005a) The disruption of *Daphnia magna* sodium metabolism by humic substances: mechanism of action and effect of humic substance source. *Physiological and Biochemical Zoology*, **78**, 1005–1016.

Glover, C.N. & Wood, C.M. (2005b) Physiological characterisation of a pH- and calcium-dependent sodium uptake mechanism in the freshwater crustacean, *Daphnia magna*. *Journal of Experimental Biology*, **208**, 951–959.

Haag, C.R. & Ebert, D. (2004) A new hypothesis to explain geographic parthenogenesis. *Annales Zoologici Fennici*, **41**, 539–544.

Haag, C.R., Riek, M., Hottinger, J.W., Pajunen, V.I. & Ebert, D. (2006) Founder event as determinants of within-island and among-island genetic structure of *Daphnia* metapopulations. *Heredity*, **96**, 150–158.

Halkett, F., Kindlmann, P., Plantegenest, M., Sunnucks, P. & Simon, J.C. (2006) Temporal differentiation and spatial coexistence of sexual and facultative asexual lineages of an aphid species at mating sites. *Journal of Evolutionary Biology*, **19**, 809–815.

Hanski, I. & Ranta, E. (1983) Coexistence in a patchy environment: three species of *Daphnia* in rock pools. *Journal of Animal Ecology*, **52**, 263–279.

Hartl, D.L. & Clark, A.G. (1997) *Principles of Population Genetics*. Sinauer Associates, Sunderland, MA, USA.

Hebert, P.D.N. & Beaton, M.J. (1993) *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis*. Helena Laboratories, Beaumont, TX, USA.

Hörandl, E. (2006) The complex causality of geographical parthenogenesis. *New Phytologist*, **171**, 525–538.

Hutchinson, G.E. (1957) Population studies – animal ecology and demography – concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415–427.

Kovel, C.G.F. & Jong, G. (1999) Responses of sexual and apomictic genotypes of *Taraxacum officinale* to variation in light. *Plant Biology*, **1**, 541–546.

Lagerspetz, K. (1955) Physiological studies on the brackish water tolerance of some species of *Daphnia*. *Archivum Societatis Zoologicae Botanicae Fennicae Vanamo, Supplement*, **9**, 138–143.

Levine, J.M. & HilleRisLambers, J. (2009) The importance of niches for the maintenance of species diversity. *Nature*, **461**, 254–257.

Lively, C.M., Craddock, C. & Vrijenhoek, R.C. (1990) Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature*, **344**, 864–866.

Lynch, M. (1983) Ecological genetics of *Daphnia pulex*. *Evolution*, **37**, 358–374.

Lynch, M. (1984) Destabilizing hybridization, general purpose genotypes and geographic parthenogenesis. *Quarterly Review of Biology*, **59**, 257–290.

Maynard Smith, J. (1978) *The Evolution of sex*. Cambridge University Press, Cambridge, UK.

Mergeay, J., Verschuren, D. & De Meester, L. (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. *Proceedings of the Royal Society B*, **273**, 2839–2844.

Mogie, M. (1992) *The Evolution of Asexual Reproduction in Plants*. Chapman and Hall, London.

Negovetic, S., Anholt, B.R., Semlitsch, R.D. & Reyer, H.U. (2001) Specific responses of sexual and hybridogenetic European waterfrog tadpoles to temperature. *Ecology*, **82**, 766–774.

Otto, S.P. & Lenormand, T. (2002) Resolving the paradox of sex and recombination. *Nature Reviews Genetics*, **3**, 252–261.

Pajunen, V.I. (1986) Distributional dynamics of *Daphnia* species in a rock-pool environment. *Annales Zoologici Fennici*, **23**, 131–140.

Pajunen, V.I. & Pajunen, I. (2003) Long-term dynamics in rock pool *Daphnia* metapopulations. *Ecography*, **26**, 731–738.

Pajunen, V.I. & Pajunen, I. (2007) Habitat characteristics contributing to local occupancy and habitat use in rock pool *Daphnia* metapopulations. *Hydrobiologia*, **592**, 291–302.

Plenet, S., Joly, P., Hervant, F., Fromont, E. & Grolet, O. (2005) Are hybridogenetic complexes structured by habitat in water frogs? *Journal of Evolutionary Biology*, **18**, 1575–1586.

Potts, W.T.W. & Parry, G. (1964) *Osmotic and Ionic Regulation in Animals*. Pergamon Press, Oxford.

Rangel, T.F.L.V.B., Diniz-Filho, J.A.F. & Bini, L.M. (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography*, **15**, 321–327.

Ranta, E. (1979) Niche of *Daphnia* species in rock pools. *Archiv Für Hydrobiologie*, **87**, 205–223.

SAS Institute (2007) *JMP*. SAS Institute, Cary, NC, USA.

Schenck, R.A. & Vrijenhoek, R.C. (1986) Spatial and temporal factors affecting coexistence among sexual and clonal forms of *Poeciliopsis*. *Evolution*, **40**, 1060–1070.

Suomalainen, E., Saura, A. & Lokki, J. (1987) *Cytology and Evolution in Parthenogenesis*. CRC Press, Boca Raton, FL, USA.

Tagg, N., Doncaster, C.P. & Innes, D.J. (2005) Resource competition between genetically varied and genetically uniform populations of *Daphnia pulex* (Leydig): does sexual reproduction confer a short-term ecological advantage? *Biological Journal of the Linnean Society*, **85**, 111–123.

Vandel, A. (1928) La parthénogénèse geographique. Contribution à l'étude biologique et cytologique de la parthénogénèse naturelle. *Bulletin Biologique de la France et da la Belgique*, **62**, 164–281.

Verduijn, M.H., Dijk, P.J.V. & Damme, J.M.M.V. (2004) Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale* s.l.): geographic parthenogenesis on a small scale. *Biological Journal of the Linnean Society*, **82**, 205–218.

Vorburger, C., Lancaster, M. & Sunnucks, P. (2003) Environmentally related patterns of reproductive modes in the aphid *Myzus persicae* and the predominance of two 'superclones' in Victoria, Australia. *Molecular Ecology*, **12**, 3493–3504.

Vrijenhoek, R.C. & Pfeiler, E. (1997) Differential survival of sexual and asexual *Poeciliopsis* during environmental stress. *Evolution*, **51**, 1593–1600.

Waervagen, S.B., Rukke, N.A. & Hessen, D.O. (2002) Calcium content of crustacean zooplankton and its potential role in species distribution. *Freshwater Biology*, **47**, 1866–1878.

Ward, R.D., Bickerton, M.A., Finston, T. & Hebert, P.D.N. (1994) Geographical cline in breeding systems and ploidy levels in European populations of *Daphnia pulex*. *Heredity*, **73**, 532–543.

Weeks, S.C. (1995) Comparisons of life-history traits between clonal and sexual fish (*Poeciliopsis*:Poeciliidae) raised in monoculture and mixed treatments. *Evolutionary Ecology*, **9**, 258–274.

Weider, L.J., Frisch, D. & Hebert, P.D.N. (2010) Long-term changes in metapopulation genetic structure: a quarter-century retrospective study on low-Arctic rock pool *Daphnia*. *Proceedings of the Royal Society B*, **277**, 139–146.

Williams, G.C. (1975) *Sex and Evolution*. Princeton University Press, Princeton.

Wilson, C.C. & Hebert, P.D.N. (1992) The maintenance of taxon diversity in an asexual assemblage: an experimental analysis. *Ecology*, **73**, 1462–1472.

Wolinska, J. & Lively, C.M. (2008) The cost of males in *Daphnia pulex*. *Oikos*, **117**, 1637–1646.