A population of sexual *Daphnia pulex* resists invasion by asexual clones

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Asexual reproduction avoids the costs associated with sex, predicting that invading asexual clones can quickly replace sexual populations. *Daphnia pulex* populations in the Great Lakes area are predominately asexual, but the elimination of sexual populations by invading clones is poorly understood. Asexual clones were detected at low frequency in one rare sexual population in 1995, with some increase in frequency during 2003 and 2004. However, these clones remained at low frequency during further yearly sampling (2005–2013) with no evidence that the resident sexual population was in danger of elimination. There was evidence for hybridization between rare males produced by asexual clones and sexual females with the potential to produce new asexual genotypes and spread the genetic factors for asexuality. In a short-term laboratory competition experiment, the two most common asexual clones did not increase in frequency relative to a genetically diverse sexual population due in part to a greater investment in diapausing eggs that trades-off current population growth for increased contribution to the egg bank. Our results suggest that a successful invasion can be prolonged, requiring a combination of clonal genotypes with high fitness, persistence of clones in the egg bank and negative factors affecting the sexual population such as inbreeding depression resulting from population bottlenecks.

1. Introduction

Sex is the dominant mode of reproduction among eukaryotes, but sex has been described as a paradox because it appears more costly than asexual alternatives. The disadvantages of sexual reproduction include the ‘cost of males’ [1–4] in which sexual females have a much reduced reproductive potential because half of their eggs develop into male offspring and the outcome of competition clearly favours asexual females in which all eggs develop into females [4,5]. Thus, assuming females are identical except for mode of reproduction, it is not yet clear how sexual populations can resist invasion and elimination by asexual genotypes [6].

One of the advantages of sex is thought to derive from differences between sexual and asexual reproduction in levels of genetic variation and genotypic diversity, and their association with fitness [6,7]. Offspring from sexual parents are generally more genetically diverse compared with offspring from asexual females, which likely explains some of the long-term benefits of sex [8]. This genetic diversity may also play a role in limiting asexual clones from invading and displacing sexual populations over the short term. For example, density- and frequency-dependent models of inter- and intraspecific competition have shown that a genotypically diverse sexual population in competition with a limited number of asexual clones can be favoured assuming that resources are limited and genotypes differ in resource usage [9–11], and these models have some experimental support [12–14]. A critical assumption of many models for the maintenance of sex is that selection favouring sexually produced offspring can operate before an invading asexual genotype completely eliminates sexual individuals. In this respect, competition models have the advantage of an immediate interaction that may delay or prevent invasion and can be combined with other models depending on more complicated genetic mechanisms that require several generations of selection for the advantages of sex to be realized [8,11,15].

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A closer examination of examples of coexistence between closely related sexual and asexual forms of a species or where sexual populations resist invasion by asexual clones can help shed light on the evolutionary advantages of sex. While theoretical models have made significant advances in understanding the advantages of sex, empirical studies lag behind [6,7,16]. Previous laboratory experiments with *Daphnia pulex* [12,13] showed that genetic variability can contribute to resisting invasion by a genetically unique clone. This study extends these observations to a natural sexual population that has been invaded by asexual clones.

The planktonic crustacean *D. pulex* represents one of several model invertebrate species (such as aphids and rotifers), with the capability for both sexual and asexual reproduction, that have made significant contributions to understanding the adaptive significance of sex [16–24]. *Daphnia pulex* in eastern North America inhabit small temporary ponds and occur as two reproductive forms (designated as asexual and sexual) that differ in the production of diapausing eggs that are shed and contained in protective ephippia, which are a modification of the maternal carapace [18–20]. Asexual forms are obligate parthenogens that produce asexual diapausing eggs, whereas sexual forms are facultative parthenogens, also known as cyclical parthenogens, producing diapausing eggs sexually (see the electronic supplementary material, S1). The sexuals incur a cost as evidenced by an early investment in males [3,5]. Although the asexuals avoid the cost of producing males, some clones occasionally produce a few males so their advantage over sexuals is somewhat reduced [3].

Asexual forms of *D. pulex* have been very successful in colonizing temporary ponds in the Great Lakes watershed. This area is dominated by a diverse array of asexual genotypes with ponds containing sexual individuals restricted to a few small areas. On a large geographical scale, 90% of 173 ponds contained asexual populations [18] and on a smaller scale, the focus of this study, 97% of 61 ponds contained asexual populations [19]. The two forms rarely coexist in the same pond [18,19]. Dominance of the asexuals in this area is probably due to a combination of the advantages of avoiding the cost of males [3,4] and the contagious spread of sex-limited meiosis-suppression genes. These genes can spread when rare males (capable of the normal meiotic production of haploid sperm) released by some asexual genotypes mate with sexual females [25–27] and generate new asexual clones that may have contributed to displacing the sexual individuals.

Mechanisms for the origin and success of new asexual clones, including genetic factors that may limit the long-term persistence of individual asexual lineages, are becoming better understood for *D. pulex* at the genomic level [27–30]. However, the ecological interactions that explain the observed widespread displacement of the sexual *D. pulex* populations by asexual clones are not fully understood. The hypothesis to explain the dominance of asexual clones is based on the contagious spread of meiosis-suppression genes following invasion of sexual populations by asexual clones producing males that can mate with sexual females [25–27,31]. This hypothesis assumes that asexual clones can disperse into sexual populations, become established and eliminate the resident sexual population through a combination of superior competition and spread of meiosis-suppressing genes. Over a several year period, we monitored the frequency of invading asexual clones in one of the rare sexual populations of *D. pulex*, examined evidence for the contagious spread of asexuality and conducted a laboratory invasion experiment to determine the factors contributing to a successful invasion. Our results suggest that under certain conditions, invasion of sexual populations by asexual clones may be delayed or resisted.

### 2. Material and methods

#### (a) Sampling and establishment of laboratory cultures

Disputed Pond is a relatively large temporary pond (approx. 100 × 20 m with a maximum depth of approx. 1 m) and is inhabited by sexual *D. pulex*, which is rare in this region based on previous surveys of ponds in the Great Lakes watershed [18,19,32]. The pond is located in Essex county, southern Ontario (latitude: 42.17447; longitude: -83.03456) next to Disputed Road in an area dominated by populations of asexual *D. pulex* and is subject to periodic flooding by the adjacent Canard River. Samples were taken from the pond using a small plankton net (mesh size 250 μm) during the beginning of the season (late April–early May) initially in 1995 and 1996 then during 10 consecutive years between 2003 and 2013 (except for 2012 when unusually high temperatures in March prevented the establishment of *Daphnia*). Generally, three or more samples were taken from the two ends and the middle of the pond and pooled. Large, brood-carrying females were transported to the laboratory and single female isolates placed in individual 100-ml plastic beakers with 60 ml of artificial zooplankton media [33]. Females were fed daily with aquarium-cultured algae consisting primarily of *Scenedesmus* sp. and *Chlorella* sp. Females released broods of parthenogenetic offspring that established a culture of genetically identical individuals, hereafter referred to as an isolate.

#### (b) Identifying mode of reproduction and assessing genetic variation

Once each isolate was established, samples were taken to determine genotypes for four enzyme loci (*Pgi*, *Pgm*, *Ldh* and *Pep*) using the methods outlined in [34]. *Ldh* and *Pep* were used to identify and distinguish asexual (*Ldh* 13 and *Pep* 12 heterozygotes, derived from a natural *D. pulex × D. pulicaria* hybrid cross [29,35]) and sexual (*Ldh* 11 and *Pep* 11 homozygotes) genotypes [36]. Mode of reproduction was also confirmed directly by raising individual females without males and noting the release of diapausing eggs (indicating asexual genotypes) or not (sexual genotypes) [36]. The enzyme loci *Pgi* and *Pgm* were chosen to quantify levels of genotypic diversity because they typically show high levels of polymorphism in *D. pulex* [19]. Furthermore, an analysis of obligate parthenogen clones based on five microsatellite loci (see the electronic supplementary material, S2 and table S1), confirmed that *Pgi* and *Pgm* were sufficiently variable to identify unique asexual clonal genotypes within and between years.

For each year, *Pgi* and *Pgm* genotypes from a sample of sexual females were tested for fit to Hardy–Weinberg equilibrium (HWE) as measured by the inbreeding coefficient (*F*<sub>IS</sub>), random association between loci and allele frequency variation among years (as measured by *F*<sub>ST</sub>) using GenePop on the Web (http://genepop.curtin.edu.au/) based on the study of Rousset [37]. Genotypic diversity was measured as the number of *Pgi*–*Pgm* genotypes (multilocus genotype, MLG) and the observed genotypic diversity (*Go*) [36] that varies from 1, when there is only a single MLG present, to a maximum value of *k*, where *k* MLGs occur at equal frequency.

Variation in ephippia production was quantified among the laboratory cultures of sexual and asexual isolates for the 2004 sample (the year with the greatest number of asexuals) after one month of growth. Each individual culture was classified as
a low or high ephippial producer (less than 10 or more than 10 accumulated ephippia, respectively) and used to test for differences in brood production between sexual and asexual isolates. Each isolate culture was also examined for the presence of males by examining 20–30 small individuals approximately weekly over a several week period.

(c) Laboratory competition experiment

Samples from several established laboratory isolates from the 2004 samples were replicated by taking three females from each culture and placing them in a test tube (16 × 300 mm) with approximately 10 ml of zooplankton media. This was used to synchronize female reproduction to obtain offspring of approximately the same size and age so that individuals used to start the competition experiment would have no reproductive disadvantages or advantages. Females in test tubes were fed cultured algae and monitored daily for brood production. Female offspring from the tubes were isolated into new test tubes. In total, 15 sexual isolates, consisting of nine different Pgi−Pgm genotypes and the two most common asexual clones found in Disputed Pond (clones 1 and 2 in table 1) were selected.

Female offspring from the synchronized test tube isolates were used to establish the competition experiment in nine 1 l glass jars containing approximately 300 ml of zooplankton media and consisting of three different treatments based on the initial starting percentage of asexual clones (75%, T75, 50%, T50, 25%; T25) with each treatment replicated three times. Each jar was established using a total of 60 females: T75 = 45 asexual + 15 sexual, T50 = 30 asexual + 30 sexual and T25 = 15 asexual + 45 sexual. The numbers of the two asexual clones were equal in T50 (15 individuals each) and differed by one individual in T75 (22 of one clone, 23 of the other) and T25 (seven of one clone, eight of the other). The sexual population consisted of one, two or three individuals from each of the 15 sexual isolates for the T75, T50 and T25 treatments, respectively. The sexual females were added to the jars on Day 0, and the asexual females were added the following day. The jars were kept at 15°C in a glass-door cabinet exposed to natural photoperiod during the summer months. Initially, 4–6 ml of aquarium-cultured algae was added to the jars twice daily (morning and afternoon). As the density increased, the amount of algae was increased to 6 ml, and finally to 9 ml twice daily to avoid food limitations. All jars received approximately equal amounts of food. No other maintenance was performed on the jars, but their general health and density were observed at least once or twice each week to ensure that Daphnia were receiving adequate food and that they were healthy. Samples were taken on days 11, 25, 52 by stirring the jar with a glass rod and pouring a 30 ml sample into a beaker. Two 30 ml samples were taken from each jar on each sampling day. The samples were counted, and each individual was classified as juvenile female, non-reproductive female, brood-carrying female, ephippial female or male. All individuals from the Day 11 sample were genotyped for the Pgi, Pgm and Ldh loci to identify the sexual and asexual genotypes. Individuals for all life-history stages from the Day 25 and Day 52 samples were also genotyped except that only a subsample of the non-reproductive and juvenile females was genotyped. Individuals from the samples were stored frozen at −70°C until genotyping. The total number of Daphnia genotyped on each sample date was 226, 347 and 237 for days 11, 25 and 52, respectively.

(d) Statistical methods

For the laboratory experiment, variation in relative frequency of asexual and sexual genotypes among the starting numbers (day 0) and the last sample date (day 52) for each treatment was tested using an analysis of deviance with quasi-binomial error and the F-test to correct for over-dispersion in R v. 3.0.2 as outlined in [38]. An analysis of deviance was also used to test for differences in the accumulated number of males and ephippial females produced between the sexuals and asexuals for each treatment. Change in genotypic diversity (Go) over the 52-day experiment was tested using aov in R with Day (0, 52 days) and treatment (T25, T50, T75) as fixed effects.

3. Results

(a) Temporal variation in frequency of asexual clones in the sexual population

A low frequency (5%) of asexual genotypes was detected in the 1995 sample based on the enzyme loci and none were detected in the 1996 sample (figure 1a). This contrasted with the sample taken in 2003 in which asexual genotypes were estimated at 16% (figure 1a). In the 2004 sample, the asexual genotypes showed a further increase in frequency to about 34%. This was followed by 8 years (2005–2013) in which the asexual genotypes were at low frequency (less than 4%) with none detected in 2006 and 2013 despite large sample sizes (figure 1a).

Fifteen different Pgi−Pgm genotypes (clones) were detected among the samples of asexual females for all years (table 1). However, only three clones (1, 2 and 6) accounted for 90% of the asexual individuals sampled (% table 1). These three common clones were also detected in multiple years (4–7 years) including two clones (1 and 6) detected in 1995, the earliest year sampled. Of the rare clones, six (4, 5, 7, 9, 11 and 12) were detected in 2 different years (either as females or males) and six (3, 8, 10, 13, 14 and 15) were only detected in a single year (table 1).

(b) Genetic diversity and life-history differences between sexual and asexual genotypes

The sample of sexual individuals across all years conformed to a typical random mating population with no significant deviation from HWE for both Pgi and Pgm (except Pgm for the 1995 sample) (electronic supplementary material, table S2) and there was no significant deviation from a random association between alleles at the two loci (test not shown). Furthermore, there was no significant differentiation in allele frequencies among the samples for Pgi (Fis = 0.0003, p = 0.373) and only a very small level of differentiation (Fis = 0.001, p = 0.044) for Pgm among all 12 years (electronic supplementary material, table S2). Genotypic diversity (Go) for the sexual isolates also showed little variation among years but was always much greater than the genotypic diversity estimated for the asexual isolates (figure 1b, p = 0.0004, Wilcoxon rank sum test). Among all years sampled, 27 Pgi−Pgm genotypes were observed for the sexual females out of the 30 possible genotypes. The three undetected genotypes all involved Pgm allele 4 with the lowest frequency. In most years, asexual females were much less common than sexual females (figure 1a) and this would obviously limit asexual genotypic diversity. However, although 95 asexual females were sampled in 2004 (table 1) and accounted for 34% of the sample (figure 1a), the asexual genotypic diversity was still much lower compared with the sexual females (figure 1b) with only three clonal genotypes making up the majority of the asexual sample (table 1). Also for the 2004
### Table 1

Number of 15 asexual *Pgi*—*Pgm* genotypes (clone, alleles 1, 2, 3, 4, 5) sampled from Disputed Pond in each of 12 years (no sample for 2012). *N* = total number of females (asexual + sexual) sampled. *M* = asexual genotypes that produced males in the laboratory. Asterisk (*) indicates the genotype for male individuals derived from asexuals sampled from Disputed Pond in 2004. All but three (clones 5, 10, 11) of the 15 *Pgi*—*Pgm* genotypes were also detected among the sexual females.

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*total asexual* | 140 | 100.00 |
sample, the frequency of laboratory cultured isolates with high ephippia production was significantly greater (G = 56.7, d.f. = 1, p < 0.001) for asexual (92%, N = 93) compared with sexual isolates (49%, N = 180).

Occasional males were detected in laboratory cultures of six of the asexual clones (1, 6, 7, 9, 12 and 14) (table 1). However, males were detected in only two of the 49 isolates for clone 1 sampled in 2004 and in only one of the six isolates for clone 6 sampled in 2003 (table 1). Individual males were also sampled from the pond in 2004 and genotyped using Ldh to distinguish males derived from asexual and sexual females. Of 198 males genotyped, only five were derived from asexual females and represented five different clones including the two most common asexual clones 1 and 2 (table 1). In addition, two males were identified as asexual clones 4 and 7 detected in the previous year (2003), and one male was detected as asexual clone 3 which was not observed among the asexual females sampled in any year. The remaining 193 males were derived from sexual females and showed no significant difference (p > 0.05) in Pgi and Pgm allele frequencies when compared with the sample of sexual females also sampled in 2004.

A small percentage of Ldh–Pep recombinant genotypes (approx. 1%) were observed as evidence for successful, but rare, mating between males produced by asexuals with sexual females (see the electronic supplementary material, S3). Further evidence for gene flow between the asexuals and sexuals from the 2011 sample was supported by genotyping the Ldh–Pep recombinants with 10 microsatellite loci previously determined to contain alleles associated with obligate parthenogenesis [29] (electronic supplementary material, table S4). In addition, two of the 15 asexual clones (table 1, clones 5 and 10) showed a banding pattern for Pgm suggesting that these clones are triploid. This was confirmed for an isolate of clone 5 based on six microsatellite loci with three alleles each and 3n nuclear DNA content (see Clone DISP1-21, table 2 in [40]). Triploid genotypes may be produced when diploid sperm (see table 2 in [26]) from males produced by asexual females combines with haploid eggs from sexual females. Mode of reproduction for the triploid genotypes in this study could not be determined due to lack of ephippia but diploid sperm combining with haploid eggs may contribute to the hybrid origin of polyploidy asexual Daphnia commonly observed in arctic regions [41].

(c) Laboratory competition experiment
The three different starting percentages of asexual clones (treatments) all showed decreases in the relative frequency of asexuals over the 52 days of the experiment (figure 2a) that was only significant between the start and end of the experiment for the T50 treatment (F1,4 = 13.53, p = 0.021). In addition, there were declines in the genotypic diversity between the start and end of the experiment for both the asexual (F1,12 = 22.50, p < 0.001) and the sexual (F1,12 = 70.15, p < 0.001) populations for the three treatments but the sexual genotypic diversity remained greater than the asexual genotypic diversity (figure 2b). The large declines in genotypic diversity followed by an increase for the sexual population (figure 2b) in the T50 and T75 are likely due to sampling effects. The only significant difference in accumulated male production (figure 3a) was in the T50 treatment (F1,4 = 48.50, p = 0.002) where the male production for the asexual population was greater (20.2 ± s.e. 1.82%) compared with the sexual population (8.51 ± 0.57%). Accumulated ephippial production was also greater for asexual females compared with sexual females (figure 3b) and significant for the T50 (F1,4 = 33.40, p = 0.004) and T75 (F1,4 = 18.86, p = 0.012) treatments following Bonferroni correction. Further details on the samples are presented in the electronic supplementary material, S4, and figures S1, S2 and S3.
increasing the opportunity for repeated influxes of diapaus-
ning eggs from diverse asexual clones since the pond is near
the mouth of Canard River that drains a relatively large
area dominated by asexuals [19]. Thus, there is a continual
supply of asexual diapauing eggs from external sources
but also from any asexual genotypes that have contributed
to the diapauing egg bank within the pond. The samples
taken from Disputed Pond over several years showed that
different asexual genotypes have invaded but remain at
very low frequencies except for the 2004 sample where
three different clones were relatively common. Samples
were collected early in the season so multiple isolates of the
same asexual genotype were likely derived from the hatch-
ing of individual diapauing eggs rather than within-season
parthenogenetic replication of genotypes after hatching of
only a few diapauing eggs. Regardless, asexual clones can
continually disperse into Disputed Pond but are not yet
increasing in frequency and becoming established despite
what appeared to be the start of a successful invasion in
2003 and 2004. In the absence of repeated immigration, the
aseuals would likely go extinct.

The lack of a successful invasion could be due to reduced
survival and reproduction of the clones caused by local
environmental conditions although this seems unlikely
since Disputed Pond is a woodland pond similar to many
ponds in the area that are collectively occupied by a gene-
tically diverse group of asexuals [19]. The inability of
aseuals to compete with the genotypically diverse resident
sexual population seems more likely, at least for the asexual
clones that have dispersed into the pond so far. Priority
effects where the much larger resident population monopol-
izes resources and may be better adapted to local conditions
has been proposed as a mechanism to explain the low estab-
ishment success of invaders despite high rates of dispersal
[44,45]. Perhaps at some future date, more competitively
successful asexual genotypes may invade and displace the
aseuals. However, given that some of the asexual clones in
Disputed Pond are capable of producing males and there is
evidence that these males have mated with sexual females,
competitively superior asexual genotypes may eventually be
produced within the pond. The contagious spread of asexuality
and the elimination of the sexual population, as originally
proposed by Hebert et al. [18], may be possible should some
of these superior asexual genotypes also produce functional
males. However, a possible impediment to the spread of
aseuality is the observed variation among males for the
production of sufficient quantities of normal haploid sperm
[26], and there is no information on the mating success of
males from asexual clones in competition with males from
sexual females.

Because they produce diapauing eggs parthenogeneti-
cally, individual asexuality genotypes are able to persist in
temporary ponds between years and the store of particular
clonal genotypes may accumulate in the diapauing egg
bank. It is uncertain if this could result in a numerical increase
in asexuality over time that could eventually displace the resi-
dent sexual population. Daphnia diapauing egg banks have
been successfully recovered and genotyped from lake sediment
cores to provide a detailed historical record of genetic changes
over many decades [46,47]. Unlike lakes, where gradual sedi-
ment deposition produces even layers containing Daphnia
ephippia over decades, temporary woodland ponds such as
Disputed Pond contain much leaf litter, fibrous root material

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Figure 3. (a) Average total percentage of males (s.e. bars) produced during the
52-day competition experiment for sexuals and asexuals for three treatments (T)
starting with 25, 50 and 75% asexual clones. (b) Average (s.e. bars) total percen-
tage of ephippial females (containing diapauing eggs) produced by sexual and
aseual females during the experiment. *p < 0.05, **p < 0.01.

4. Discussion

The theoretical costs of sex imply that there are distinct advan-
tages for asexual mutants arising within sexual lineages [6].
This appears to be the case for D. pulex where asexuals
dominate temporary ponds throughout the Great Lakes area,
consistent with the historical invasion and displacement of
sexual populations by asexual clones [18,19]. Furthermore,
the observed high genotypic diversity strongly suggests that
the success of asexual clones is facilitated by meiosis-
suppressing genes transmitted by some clones producing func-
tional males that mate with sexual females resulting in the
transformation of sexual genetic diversity into a diverse
group of asexual genotypes [26]. Although this general process
can explain the success of the asexual D. pulex genotypes over
the longer time period [27] since the area was de-glaciated
approximately 10,000 years ago, this study suggests that
particular genetic and/or ecological factors are required for
a successful invasion of individual sexual populations by
aseual clones.

Invasion of asexual clones into the rare geographically
isolated sexual D. pulex populations could be avoided or
delayed due to limited opportunity for dispersal into these
ponds [42]. Caceres & Soluk [43] found that passive dispersal
(wind and rain) or dispersal by animal vectors (birds, frogs
and insects) of Daphnia diapauing eggs into new ponds
was delayed relative to other pond invertebrate species.
Although aerial dispersal of ephippia containing diapauing
eggs has been reported for Daphnia [43], movement of hydro-
phobic ephippia during flooding is probably much more
effective. Disputed Pond is subjected to periodic flooding

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and often dry completely during the summer preventing a similar historical reconstruction. Ephippia with diapausing eggs are deposited during the spring growing season and some hatch the following year but how much egg bank storage contributes to subsequent years is unknown. Nevertheless, even short-term storage of diapausing eggs allows asexuals to persist in the pond and may allow particular asexual clones to increase in frequency in subsequent years.

Mergay et al. [46] used the diapausing egg bank to reconstruct a remarkably rapid (60 years) invasion of African lakes by a single clone of asexual Daphnia of North American origin. The invading clone was a D. pulex × D. pulicaria hybrid, as are those described in this study with the possible benefits of hybrid vigour, which displaced the genetically diverse native sexual D. pulex population. Clearly, successful invasion by asexuals does not necessarily require genetic variation, also found for other invading asexual species [48,49] and genetically variable sexual populations are not immune to such invasion [46]. For these studies, it is not known whether the invasion success of a particular clonal genotype was just one of many other genotypes that were unsuccessful. However, ecological factors such as changes in land-use patterns, eutrophication and fish introductions can contribute to the competitive success of invading Daphnia species [50] including the D. pulex clone in the African lakes where these genotypes are adapted to the changed conditions.

Previous laboratory experiments with D. pulex [13] demonstrated that genetic diversity confers a competitive advantage, expressed as a greater reproductive rate, due to resource partitioning among the genetically diverse group but not for the genetically uniform clone. Further experiments [12] showed that the competitive advantage of resource partitioning for a genetically diverse population was sufficient to resist invasion by a genetically uniform clone. However, these experiments only considered the reproductive rate as measured by all-female parthenogenetic brood production. The competitive ability of asexual D. pulex based solely on avoiding the cost of males would also be reduced for asexual genotypes that produce males as was observed for some of the clones invading Disputed Pond. In addition, increased investment in diapausing eggs for the asexuals relative to sexual females can further reduce within-season competitive ability by decreasing parthenogenetic brood production. These life-history differences could account for the failure of the two asexual clones to outcompete the genotypically diverse sexuals in the short-term laboratory experiment and may similarly explain the lack of increase in frequency of these asexual clones in Disputed Pond. Given the short growing season, both sexual and asexual forms have to trade-off parthenogenetic brood production, which numerically increases a genotype within a season, with diapausing egg production required for persistence between years. A greater investment in diapausing egg production by the asexuals may mean a delay in invasion success as diapausing eggs accumulate in the egg bank.

In summary, genotypically diverse asexual D. pulex have successfully spread over a wide geographical area predicting that any rare sexual population, such as the one in Disputed Pond, will eventually be invaded and replaced with asexual clones. A diversity of asexual clones has invaded Disputed Pond but sampling over an 18-year period has yet to show any immediate threat to the resident sexual population. One or more of the invading clones may eventually increase in frequency and displace the sexual population through a combination of direct competition, a numerical increase in the diapausing egg bank and repeated dispersal from external sources. Successful invasion may require asexual clones from external sources with superior competitive ability or new clones generated by mating between males produced by the invading asexual clones and resident sexual females. Successful invasion may also depend on periodic reductions in the size of the sexual population resulting in reduced fitness through inbreeding depression, which would favour more outbred asexual clones [51,52]. A recent genomic analysis supports the importance of mating between males from asexual clones with sexual females for generating new asexual genotypes that have the potential for superior fitness, and also for rescuing asexual genomes from deterioration due to gene conversion and deletion [27]. However, this study shows that the time frame of the invasion may be prolonged and, as found for other examples [48,53–55], detailed observations of the interaction between ecological and genetic factors are required to fully understand the invasion process.

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