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Effects of plant sex on range distributions and allocation to reproduction

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Summary

- Despite an abundance of theory, few empirical studies have explored the ecological and evolutionary consequences of sex. We used a comparative phylogenetic approach to examine whether transitions between sexual and asexual reproduction are associated with changes in the size and distribution of species' geographical ranges, and their investment in reproduction.
- Here, we reconstructed the phylogeny of the genus *Oenothera* sections *Oenothera* and *Calylophus* (Onagraceae), which contain 35 sexual and 30 functionally asexual species. From each species, we collected data on the geographical distribution and variation in plant traits related to reproduction.
- Functionally asexual species occurred at higher latitudes, but did not differ in range size, compared with sexual species. Transitions to asexuality were associated with decreased investment in floral structures, including the length of petals, floral tubes and styles. Decreased anther size and increased seed size within asexual species also suggest altered allocation to male and female fitness.
- The observed range shifts are consistent with superior colonization of environments by asexual species following glaciation, and the observed changes in reproductive allocation support predictions made by models relating to the evolution of selfing. Our results suggest that the evolutionary consequences of asexual reproduction might be less restrictive than previously thought.

Introduction

Biologists have long sought explanations for the prevalence of sexual reproduction in nature (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Salathé *et al.*, 2008; Otto, 2009), whereas the consequences of sexual and asexual reproduction for the ecology and evolution of populations have been the focus of relatively few empirical investigations. This problem is particularly relevant to flowering plants, which exhibit a near-continuum between sexuality and asexuality (Barrett, 2002; Goodwillie *et al.*, 2005; Whitton *et al.*, 2008). Here, we seek to understand how variation in the mode of sexual reproduction influences the size and distribution of plant species' ranges and the evolution of investment in reproductive structures. We focus on the extremes of this continuum by comparing plant species that exhibit partial or obligate cross-pollination with closely related species that are functionally asexual.

Compared with research on the evolution and ecology of selfing, there is relatively little theoretical or empirical literature addressing the consequences of asexuality for species' distributions and allocation to reproductive structures (Glesener & Tilman, 1978; Lynch, 1984; Mogie, 1992; Noirot *et al.*, 1997). However, because many of the effects of asexuality are likely to be similar to those of obligate selfing, we can draw on the extensive plant mating system literature to make tentative predictions. For example, whether a species is obligately outcrossing or selfing is expected to have consequences for the size and distribution of its range. Self-compatibility provides plants with reproductive assurance that facilitates the colonization of new environments (Baker, 1955). This effect has led some to predict that

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selfing species can more easily form new populations at the borders of their ranges, facilitating range expansion and leading to larger ranges than those of obligately outcrossing species (Lowry & Lester, 2006; Randle *et al.*, 2009). Species that produce seeds asexually are expected to experience similar ecological advantages over outcrossing species (Stebbins, 1950; Maynard Smith, 1978; Bierzychudek, 1985). On the other hand, a loss of sex can also decrease the amount of genetic variation and increase genetic load within populations (Otto & Lenormand, 2002), which might prevent adaptation to new environments. If this effect is large, it may counteract the colonization advantage of asexuality. Thus, changes in geographical range associated with transitions between sexuality and asexuality may depend on the relative importance of colonization ability and adaptive potential. Macroevolutionary comparative studies, such as those undertaken here, have the potential to determine whether changes in sexuality are consistently correlated with increases or decreases in range size across species.

Increased selfing and asexuality are also expected to cause shifts towards higher latitudes, although the mechanisms underlying such shifts remain a point of contention. For example, several authors have argued that biotic interactions (e.g. competition, predation) are less predictable at lower latitudes, selectively favoring sexual modes of reproduction, whereas asexual populations outcompete sexual populations at higher, more biotically predictable latitudes (Glesener & Tilman, 1978; Bell, 1982). Lynch (1984) questioned the logic and data supporting this mechanism and proposed that the evolution of asexuality selects for general-purpose genotypes that occupy a greater range of environments than do sexual taxa. By this logic, asexual lineages occupy larger ranges, including higher latitudes, because the abiotic environments of these areas are more extreme (e.g. fluctuations in cool temperatures can lead to pollinator failure at high latitudes), which Lynch (1984) claims are most amenable to an asexual mode of reproduction. A third possibility is that sexual and asexual lineages do not differ fundamentally in their niche requirements, but the colonization advantage of asexuals allows them to expand more quickly during periods of range expansion, such as following glaciation (Bierzychudek, 1985; Law & Crespi, 2002). Although all three mechanisms share the prediction that asexuals should occur at higher latitudes, there are surprisingly few studies that have compared the latitudinal distributions of sexual and asexual plants (Bierzychudek, 1985; Silvertown, 2008). Although the results of these studies support predicted patterns, the identification of the causal effect of the observed patterns is made difficult because variation in the mode of reproduction is often confounded by variation in ploidy (Bierzychudek, 1985).

Research on the evolution of mating systems also makes predictions about the effects of selfing rate on the evolution of allocation to male vs female reproduction in hermaphroditic organisms (Charnov, 1979; Charlesworth & Charlesworth, 1981, 1987). Specifically, as selfing increases from obligate outcrossing to complete selfing, we expect to observe decreased investment in male reproduction (e.g. pollen) and in structures involved in the attraction of pollinators (e.g. petals and floral tubes), and increased investment in female reproduction (e.g. seed number and size) (Queller, 1984; Charlesworth & Charlesworth, 1987). These patterns are expected because highly selfing individuals can fertilize their ovules with less pollen and therefore are able to divert unused resources to investment in seeds (Charlesworth & Charlesworth, 1981). In addition, they have little need to attract pollinators to export pollen (Bell, 1985). Experimental tests of this aspect of sex allocation theory within (Schoen, 1982; Mazer *et al.*, 1999; but see Mazer *et al.*, 2007) and between (Cruden & Jensen, 1979; Ritland & Ritland, 1989; Parker *et al.*, 1995; French *et al.*, 2005) species generally support predictions. However, tests of this theory in a macroevolutionary context using molecular-based estimates of selfing rates and phylogenetically explicit analyses are rare (Michalski & Durka, 2009). Similarly, we might predict that, when there is a transition from sexual to asexual reproduction, there would be similar shifts in allocation to reproduction. Like highly selfing individuals, asexual individuals do not need to export pollen and thus do not receive the benefits from the production of large amounts of pollen or costly pollinator attraction structures. However, this prediction might not be realized if asexuality results in greatly depleted genetic variation and thus decreased evolvability (Hill & Robertson, 1966; Barton & Otto, 2005).

The evening primroses (genus *Oenothera*, Onagraceae) offer an ideal system to investigate the ecological and evolutionary effects of sexual vs asexual reproduction. This genus has undergone approximately 20 transitions between sexual (i.e. recombination and segregation) and functionally asexual (no recombination or segregation) reproduction (Johnson *et al.*, 2009). This functional asexuality has arisen because of a well-studied genetic system called 'permanent translocation heterozygosity' (PTH), which occurs in as many as eight plant families (Cleland, 1972; Holsinger & Ellstrand, 1984; Harte, 1994). Like apomixis, PTH reproduction typically results in seeds that are genetically identical to one another and to their parent plant, but, unlike apomictic species, PTH species undergo all stages of meiosis and fertilization. Functional asexuality arises because of three phenomena (Cleland, 1972; Harte, 1994): First, chromosomal translocations alter chromosomal homology, so that chromosomes form a complete ring at metaphase I which prevents recombination (Cleland, 1972; Rauwolf *et al.*, 2008). Second, segregation is prevented by a balanced lethal mortality of gametes, such that one haploid set of chromosomes always segregates together and passes through the ovules, whereas the other haploid set always passes

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through the pollen (Cleland, 1972; Rauwolf *et al.*, 2008). Third, PTH species typically self-fertilize by dehiscing pollen onto receptive stigmas before the flowers open. In essence, seeds are formed by pulling apart the parental genome and putting it back together without recombination or segregation. An additional advantage of studying PTH reproduction instead of other forms of functional asexuality is that the former typically exhibits the same ploidy level as sexual relatives, whereas apomictic species are typically polyploid and their sexual relatives are either diploid or of a different ploidy level, such that variation in sex and ploidy are confounded (Stebbins, 1950; Whitton *et al.*, 2008).

In this study, we used a comparative phylogenetic approach to examine the consequences of changes in reproductive mode on species' ranges and the evolution of plant traits in the genus *Oenothera*. We focused on section *Oenothera* within the genus *Oenothera*, because PTH is best characterized in this section and the clade contains many sexual and PTH species (Cleland, 1972). We first estimated the phylogeny of this clade using two nuclear regions and one plastid region, and then examined three specific questions. First, is PTH reproduction associated with higher latitudes and larger range sizes? Second, do PTH species exhibit predicted decreases in investment to structures related to male reproduction and attraction of pollinators, and increased investment in female reproductive parts? Third, does PTH reproduction affect the evolution of traits not directly related to reproduction (i.e. plant growth and light capture), which would not be predicted to adaptively change with a loss of sex, and which therefore serve as a control for the reproductive traits examined?

Materials and Methods

Experimental system and data collection

The genus *Oenothera* is native to North and South America, with many species establishing populations in the last several hundred years on all habitable continents. Plants typically occur in open habitats, such as prairies, grasslands, beaches and recently disturbed areas. We focused on species in sections *Oenothera* and *Calylophus* because recent phylogenetic results have indicated that these sections form a monophyletic clade within the genus *Oenothera* (Ford & Gottlieb, 2007; Johnson *et al.*, 2009), and because this clade contains the largest number of PTH species as well as their sexually reproducing relatives (Raven, 1979; Holsinger & Ellstrand, 1984; Johnson *et al.*, 2009). Flowers in these sections comprise four yellow petals (sometimes flushed with red) and conspicuous floral tubes, which are involved in attracting moths at night and Hymenoptera during the day (Clinebell *et al.*, 2004; M. Johnson, pers. obs.). Sexual species have chasmogamous flowers, typically with stigmas conspicuously exerted beyond the anthers, whereas the stigmas of PTH species are usually surrounded by anthers and flowers are occasionally cleistogamous. Fruits dehisce and release seeds with no specialized dispersal structures directly beneath the parent plant. There are no clear life history differences between closely related sexual and PTH species, and they can, in some instances, be found growing immediately adjacent to one another (M. Johnson, pers. obs.).

We focused our sampling on species from taxonomic subsections in which both PTH and sexual reproduction were prevalent. Within section *Oenothera*, we sampled 31 of the 60 species from subsections *Candela*, *Munzia*, *Oenothera* and *Raimannia*, and three of the five species in section *Calylophus* (Wagner *et al.*, 2007). In *Oenothera*, species are delimited on the basis of a combination of morphology and molecular systematics, as well as post-mating barriers and cytological behavior determined by experimental crosses (see Wagner *et al.*, 2007). The delimitations of subspecies in *Oenothera* is highly subjective and has been more intensely scrutinized in some clades than others. We therefore take a conservative approach by recognizing only a single taxon per species and concatenating the trait data across subspecies. In this way, we used the same objective criterion for delimiting taxa and collecting data for all species. We sampled eight outgroup species, including four additional *Oenothera* spp. and four species from other genera within the Onagraceae.

Range size and latitude

The size of species' native ranges and latitude were measured from detailed distribution maps showing the localities of individual populations represented by herbarium specimens. We used Towner (1970) as our source for section *Calylophus*, Dietrich & Wagner (1988) for subsections *Candela* and *Raimannia*, Dietrich *et al.* (1997) for subsection *Oenothera* and Dietrich (1977) for subsection *Munzia*. When collecting data, we combined all subspecies into a single taxonomic unit and measured a species' range size using Google Planimeter (http://www.acme.com/planimeter) as the area of the minimum convex polygon that circumscribed all localities (Gaston, 1996). When a single occurrence of a species was more than 500 km from the nearest edge of the polygon that excluded the singleton population, we deemed it to be a non-native introduction and excluded it from the calculations. Introduced populations outside of the native range (e.g. *O. biennis* in Europe) were similarly excluded. In cases in which multiple subspecies have been described within a species, we combined subspecific range data by circumscribing the minimum convex polygon around all points, unless the edges of polygons around each individual subspecies were more than 500 km apart (e.g. *O. drummondii* ssp. *drummondii* and *O. drummondii* ssp. *thalassaphila*); in this case, we added the individual polygon areas. We also removed *O. glazioviana*

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from analyses on range size and latitude because this species arose *de novo* in Europe within the last 500 yr and the species' distribution is probably in flux. The localities of populations shown in distribution maps were also used to determine the highest, lowest and median latitude (calculated as the midpoint of the highest and lowest latitudes) of the native range for each species. Degrees of latitude were measured using Google Earth (Google, Mountain View, CA, USA).

Measurement of plant traits

We collected data on plant traits using the same references as were used for range size. We focused on floral and reproductive traits (petal length, floral tube length, style length, epipetalous filament length, anther length, capsule length, seed length), as there are clear predictions about how variation in the mode of sexual reproduction influences investment in these structures. As an internal control and a basis for comparison, we also measured traits related to plant growth (height) and resource acquisition (leaf length, leaf width), because a loss of sex is not expected to directly select for changes in these traits, as sexual and PTH sister taxa typically inhabit the same types of environment. All of these data were based on measurements collected from multiple plants found in many populations across species' ranges and were reported by the authors as the range in trait values recorded (i.e. minimum and maximum values), from which we obtained the median trait value by calculating the midpoint value of the reported range for each species. As before, we combined all subspecies into a single taxonomic unit, although, for accuracy, our phylogeny shows the specific taxon that was sequenced (Fig. 1). The raw trait data used in analyses are provided in Table S1(see Supporting Information). To verify the accuracy of published measurements, we grew one to eight replicate plants (average, 3.5) to flowering from a subset of the species included in our analyses (eight sexual, six PTH), and measured all of the plant traits listed above, except for anther length, capsule length and seed length. We then performed pairwise correlations between the midpoint values recorded from the literature and species' mean values based on our own measurements of multiple plants within each species. All trait measurements were highly positively correlated (Pearson r values, 0.74–0.98; $P < 0.03$ for all traits; $n = 9$–14 species).

Molecular sequence data and phylogeny estimation

To estimate the phylogeny of sections *Oenothera* and *Calylophus*, we combined existing datasets from Genbank for

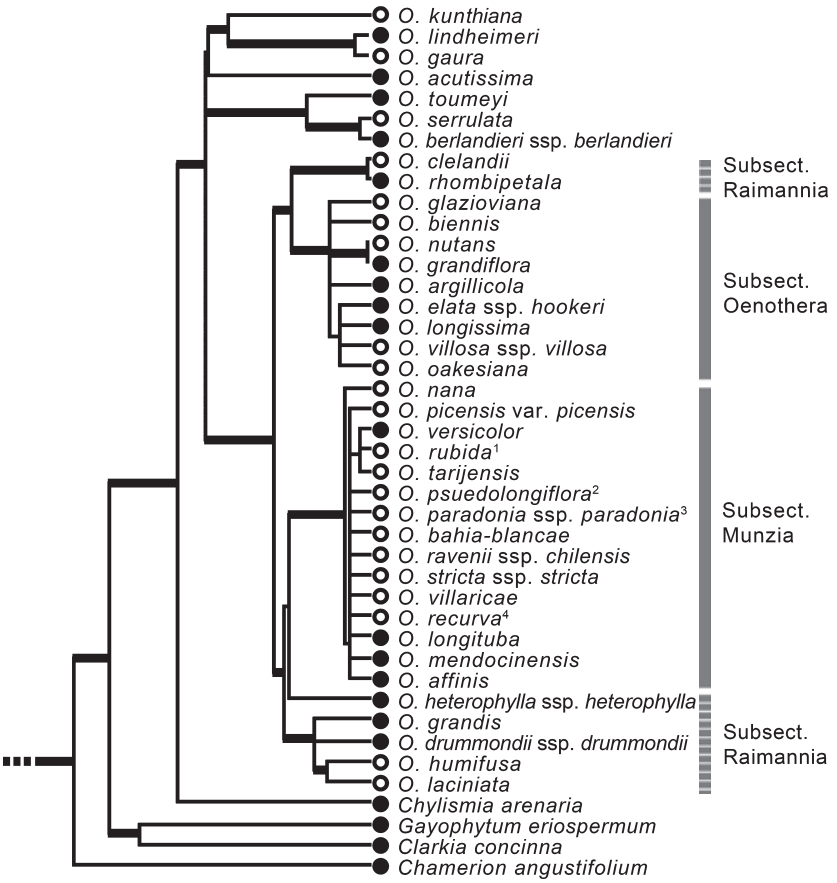

Fig. 1 Phylogeny of *Oenothera* section *Oenothera*. The tree from combined maximum likelihood analysis of nuclear (ITS, *pgiC*) and plastid (*trnL-trnF* spacer) sequences was ultrametricized (as shown) for comparative analyses. Bold branches have more than 50% bootstrap support and 100% posterior probability. Open circles, permanent translocation heterozygote (PTH) taxa; filled circles, sexual taxa. When PTH was polymorphic within a species, we denoted a species as PTH if more than 50% of individuals sampled in taxonomic studies exhibited a ring of 14 chromosomes. *Fuchsia cyrtandroides* and *Ludwigia peploides* (not shown) were used as outgroups in all analyses. Monophyletic subsections are shown as filled gray bars and nonmonophyletic subsections as gray dashed bars. Note: [1]*O. rubida* Rusby = *O. sandiana* Hasskarl (Wagner *et al.*, 2007); [2]*O. pseudolongiflora* Dietrich = *O. paradonia* Munz (Wagner *et al.*, 2007); [3]*O. paradonia* ssp. *paradonia* Dietrich = *O. paradonia* Munz (Wagner *et al.*, 2007); [4]*O. recurva* Dietrich = *O. tarijensis* Dietrich (Wagner *et al.*, 2007). We retain the original names of these taxa as this phylogeny provides an independent test of whether these taxa are separate species.

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the plastid *trnL-trnF* spacer region (Taberlet *et al.*, 1991), the single copy nuclear region [introns 1–6 (plus exons)] of the cytosolic isozyme phosphoglucose isomerase (*PgiC*) (Ford & Gottlieb, 2007) and the nuclear ribosomal internal transcribed spacer (ITS) region (Hoggard *et al.*, 2004; Levin *et al.*, 2004) (Table S2, see Supporting Information). To expand the dataset, we sequenced *trnL-trnF* and ITS from 29 additional species and *PgiC* from 39 species (Table S2) using standard PCR conditions (Ford & Gottlieb, 2003; Hoggard *et al.*, 2004; Levin *et al.*, 2004). In total, our taxon sampling encompassed 34 species within sections *Oenothera* and *Calylophus* plus eight outgroup taxa (Fig. 1; Table S2).

PCR products were directly sequenced when possible with the primers used to isolate the gene. Sequencing was performed using an ABI 3730xl DNA sequencer and BigDye protocols (Applied Biosystems, Foster City, CA, USA). Allelic length variants were detected at *PgiC* for a small number of species. In these cases, we cloned the PCR products using the pGEM-T Easy Vector System (Promega, Madison, WI, USA) and sequenced four to six clones per product to identify the alleles. No individuals revealed more than two alleles, consistent with previous results that showed that *PgiC* is present as a single copy within *Oenothera* (Ford & Gottlieb, 2007). Multiple sequences from each species were initially included in phylogenetic analyses, but, as alleles from the same species consistently clustered together, we randomly selected a single representative sequence for each species for the final dataset.

We tested for conflicting phylogenetic signals among the three independent datasets using the incongruence length difference (ILD) test (Farris *et al.*, 1994) implemented in PAUP4.0b10 (Swofford, 2002). The tests were conducted using 500 replicate partitions, each subjected to heuristic searches with simple taxon addition, tree bisection–reconnection (TBR) branch swapping and keeping no more than 500 trees per replicate. ILD tests were conducted for each pair of genes, and pairs returning nonsignificant *P* values were combined for the final analyses.

Maximum likelihood and Bayesian analyses were used for phylogenetic inference. The model of substitution for each dataset was selected using hierarchical likelihood ratio tests in PAUP4.0b10 (Swofford, 2002). Likelihood scores were calculated using a neighbor-joining tree for a range of models (JC, K2P, HKY, HKY + Γ, HKY + Γ + I, GTR + Γ and GTR + Γ + I) (Swofford *et al.*, 1996), and the best-fitting model was used for subsequent likelihood and Bayesian analyses.

Bayesian analyses were performed on the combined dataset in MrBayes version 3.1.1 (Ronquist *et al.*, 2005) with the data partitioned by gene and, for *PgiC*, by coding and noncoding regions. Model parameters were unlinked across partitions and analyses were run for 3 million generations, sampling every 100 generations. We conducted two independent runs, each comprising four linked chains with temperature set to 0.05, which gave acceptance rates for swapping between adjacent chains in the range of 10–70%. The first one-third (10 000) of trees were discarded as 'burnin', and the remaining 'post-burnin' trees were used to construct consensus trees. Convergence of the runs was judged by examining convergence diagnostics in the sump output, and by comparing consensus trees from the two independent runs.

To provide a single best estimate of topology and branch lengths for comparative analyses, we undertook combined likelihood analyses with RAxML-7.0.4 (Stamatakis, 2006). A GTR + Γ model was used and model parameters were estimated independently for each of the four data partitions (ITS, *trnL-trnF* spacer, *PgiC* introns and *PgiC* exons). As an additional measure of support for the relationships inferred, we conducted parsimony bootstrap analyses in PAUP4.0b10 (Swofford, 2002). Each of the 500 bootstrap replicates was subjected to heuristic searches with 10 random sequence additions, TBR branch swapping and 'maxtrees' set to 100.

Comparative phylogenetic analyses

We used phylogenetic regression methods to test for the effects of sexual vs PTH reproduction on the evolution of plant traits (Grafen, 1989; Martins & Hansen, 1997; Garland *et al.*, 2005). Specifically, we used restricted maximum likelihood to estimate parameters that test for the association between plant sex and variation in plant traits, whilst taking into account the phylogenetic relatedness of species and a specific model of trait evolution across the tree (Lavin *et al.*, 2008). All analyses utilized median trait values and the variance–covariance matrix calculated from the single maximum likelihood phylogeny inferred by RAxML (Stamatakis, 2006) and ultrametricized using NPRS in TreeEdit (Rambaut & Charleston, 2002), as shown in Fig. 1. Analyses were performed in MATLAB 7.7 (The Mathworks, Natick, MA, USA) using the program REGRESSIONv2.m as described by Lavin *et al.* (2008). PTH and sexual reproduction were coded as 0/1 dummy variables, and Akaike Information Criterion (AIC) values were used to compare the adequacy of three models of trait evolution: (1) traits that evolve independent of phylogeny, such that their relationship can be portrayed as a star phylogeny [ordinary least-squares (OLS) regression]; (2) traits that evolve according to a random walk Brownian motion-like process across the phylogeny (phylogenetic generalized least-squares, PGLS); and (3) trait evolution that is constrained through time according to an Ornstein–Uhlenbeck (OU) process that resembles the effects of stabilizing selection towards an optimum (Martins & Hansen, 1997; Blomberg *et al.*, 2003). The relative fits, parameter estimates and statistics for all analyses are provided in Table S3 (see Supporting Information). Across all analyses, the OU

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model provided either the best fit to the data (i.e. [AIC_{OLS} or AIC_{PGLS}] − [AIC_{OU}] > 2) or a fit that was equivalent to an OLS regression model ($AIC_{OLS} - AIC_{OU} < 2$); the Brownian motion model always provided a poor fit to the data. The statistical significance of the effect of plant sex on plant traits was assessed using a *t*-test and parametric bootstrapping, but, as both methods resulted in identical conclusions, we only report the former.

Results

Phylogenetic estimation

Our three-gene dataset provided over 400 informative characters for resolving the relationships between *Oenothera* section *Oenothera* and related lineages (Table S4, see Supporting Information). Analyses of congruence showed no strong conflict among datasets for all pairwise ILD tests ($P > 0.40$), and therefore we combined datasets for phylogenetic analyses.

Maximum likelihood analysis revealed several major lineages within the monophyletic *Oenothera* section *Oenothera*, and many of these lineages received high bootstrap support (Fig. 1). The North American subsection *Oenothera* and South American subsection *Munzia* formed well-supported clades, whereas subsection *Raimannia* appeared to be paraphyletic. Bayesian analyses recovered similar topologies with high posterior probabilities for the clades which received high bootstrap support (> 50%, Fig. 1).

Geographical distribution and range size

Consistent with predictions, the median latitude of a species' range was *c.* 3.25° or 333 km higher for PTH than for sexual species (Fig. 2a), such that PTH species typically occurred at latitudes closer to the poles in both northern and southern hemispheres (Table 1). The magnitude of this shift was equivalent to 21% of the latitudinal range of an average *Oenothera* species. PTH species also exhibited a trend for the edges of species' ranges to be at higher latitudes, but these effects were not significant (lower latitudinal range edge, $P = 0.23$; higher latitudinal range edge, $P = 0.13$).

PTH species exhibited 72% larger mean geographical range sizes than sexual species, and the species with the four largest range sizes all employed PTH reproduction (Fig. 2b). However, this difference in mean range size was not statistically significant (Table 1), because both reproductive strategies contained species that were widespread as well as species that were geographically restricted.

Plant trait evolution

A breakdown in sexual reproduction had a pronounced effect on the size of floral traits involved in the attraction of insects and the positioning of male and female sexual organs (Table 1, Figs 3,4). PTH reproduction was associated with a 47% decrease in median petal length (Figs 3a,4) and a 22% decrease in floral tube length (Figs 3b,4), compared with sexual species (Table 1). The reduction in petal length corresponded to flowers of PTH species having approximately one-quarter the surface area of sexual species. Flowers also experienced a repositioning of sexual organs, as we detected a 31% reduction in the length of styles and a 26% decrease in the length of epipetalous filaments in PTH compared with sexual species (Table 1, Fig 3c). The effect of these reductions altered the herkogamy seen in sexual spe-

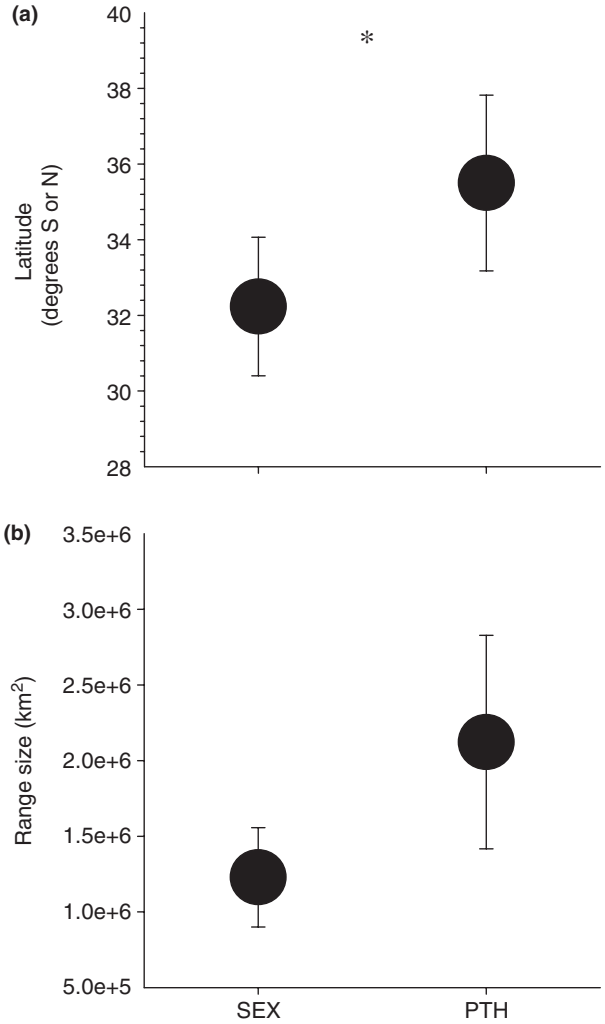

Fig. 2 Median latitude (a) and geographical range size (b) in sexual and permanent translocation heterozygote (PTH) species. Median latitude was determined for each species by taking the midpoint value between the lowest latitude at which a species was recorded and the highest latitude at which a species was recorded in its native range. The geographical range size was determined for each species by taking the area of the minimum oblique polygon that encompassed a species' recorded locations (see Materials and Methods). Phylogenetically adjusted mean values are shown with whiskers indicating 1SE of the raw species' mean values.

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Table 1 The effects of sex vs permanent translocation heterozygote (PTH) reproduction on the evolution of geographical range size and plant traits

Trait	df	t	P	r^2
Latitude	30	**7.93**	**< 0.001**	0.68
Range size	30	1.48	0.149	0.07
Petal length	31	**−6.50**	**< 0.001**	0.58
Floral tube length	31	**−6.61**	**< 0.001**	0.58
Style length	31	**−3.19**	**0.003**	0.25
Anther length	31	**−4.05**	**< 0.001**	0.35
Filament length	31	**−2.59**	**0.015**	0.18
Capsule length	31	−2.01	0.053	0.12
Seed length	31	**11.70**	**< 0.001**	0.82
Leaf length	30	−0.10	0.923	0.00
Leaf width	30	1.08	0.290	0.04
Plant height	31	−1.08	0.289	0.04

Each trait was modeled across the phylogeny according to an Ornstein–Uhlenbeck process which provided the best fit to the data according to the Akaike Information Criterion (AIC) statistic for all but two traits, where a nonevolutionary model provided an equivalent fit. To test for differences in the phylogenetically adjusted mean values between sexual and PTH species, we report t-statistics, the associated P value and the explanatory power for the effect of sex (r^2). Statistically significant P values ($P < 0.05$) are shown in bold.

cies, in which stigmas typically extend beyond the anthers, such that stigmas in PTH species typically surround and touch the anthers (Towner, 1970; Dietrich, 1977; Dietrich & Wagner, 1988; Dietrich *et al.*, 1997).

PTH reproduction was also associated with the evolution of altered allocation to components of male and female reproduction (Table 1). PTH species exhibited 38% shorter anthers than sexual species (Fig. 3e), suggesting that there has been evolution for decreased investment in male reproduction via reduced pollen production, as anther size correlates with pollen production (Ritland & Ritland, 1989). We also found that fruit capsules were 8% shorter in PTH species, and the length of individual seeds was 9% larger (Table 1; Fig. 3f). By contrast, traits that were not directly related to pollination and reproduction (i.e. plant height, leaf length and leaf width) did not differ significantly between sexual and PTH plant species (Table 1).

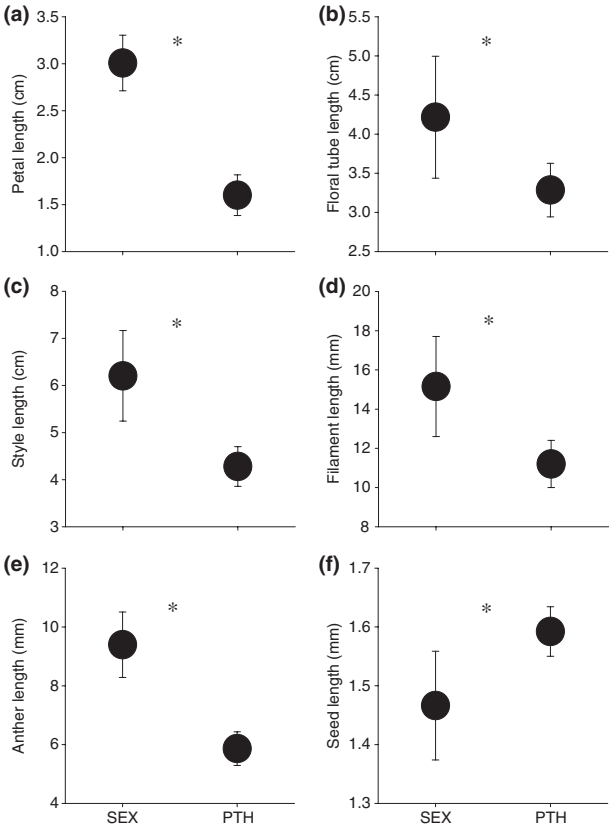

Fig. 3 Floral and reproductive trait values for sexual and permanent translocation heterozygote (PTH) plant species. Phylogenetically adjusted mean values are shown with whiskers indicating 1SE of the raw species' mean values for: (a) petal length; (b) floral tube length; (c) style length; (d) filament length; (e) anther length; (f) seed length. The upper range value for the seed length of PTH species is obscured by the symbol showing the overall mean. *Differences between PTH and normal sexual reproduction that were significantly different at $P < 0.05$ using parametric and bootstrap statistics.

Discussion

We found that repeated losses of recombination and segregation have had pronounced effects on the ecology and evolution of *Oenothera* species. Most notably, PTH species occurred at higher median latitudes than sexual relatives (Fig. 2a), consistent with predictions that asexual species are better able to establish and persist in extreme environments and/or colonize areas following glaciation. PTH species also evolved decreased investment in structures that attract pollinators and increase male fitness, whereas there was an increase in investment of at least one trait (seed size) related to investment in female reproduction. These results largely support theoretical predictions, many of which were originally proposed as an investigation into the effects of selfing rates, but which also provide a useful framework for understanding the ecological and evolutionary consequences of asexuality.

Geographical distribution

We found support for the predicted pattern that asexual taxa are found at higher latitudes than are sexual taxa, with the median latitude for PTH species 3.25° or 333 km higher than that of sexual species. The only previous phylogenetically explicit test of this pattern that we are aware of focused on a comparison of 40 species containing both sexual and asexual lineages (Bierzychudek, 1985). That study also found that asexual lineages occurred at higher latitudes, but the interpretation of the results was complicated by the

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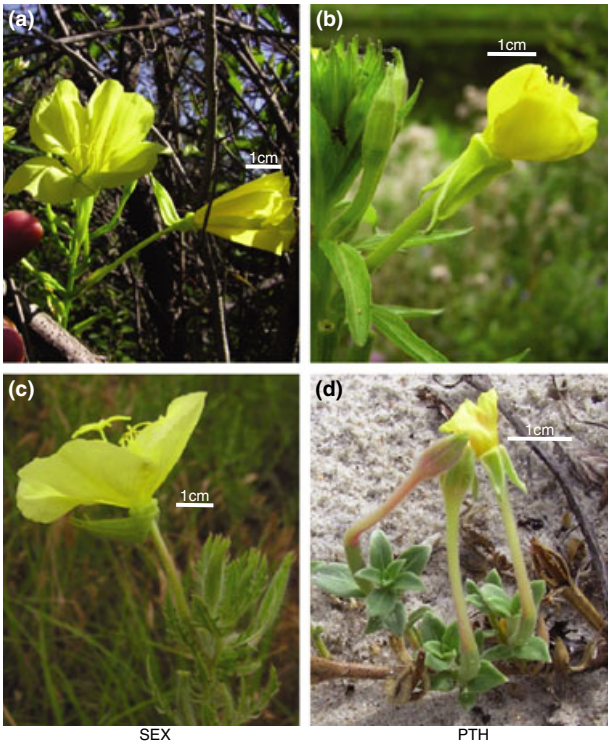

Fig. 4 Flowers of sexual and permanent translocation heterozygote (PTH) plant species. *Oenothera grandiflora* (sexual) (a) and *O. biennis* (PTH) (b) are closely related taxa in subsection *Oenothera*. *Oenothera grandis* (sexual) (c) and *O. humifusa* (PTH) (d) are closely related taxa in subsection *Raimannia*. Photo credit: M. Johnson.

fact that asexual lineages typically had higher ploidy than their sexual counterparts; ploidy variation itself can influence species' distributions (Lowry & Lester, 2006). Therefore, our results provide the first unequivocal evidence supporting the idea that a breakdown in sexual reproduction is sufficient to cause species' range shifts (but see Silvertown, 2008).

The mechanistic basis for the observed range shifts is more difficult to discern. One mechanism proposes that greater uncertainty in the biotic environment at lower latitudes selects for sexual reproduction, whereas higher latitude sites are argued to be more predictable, which is thought to favor asexual reproduction (Glesener & Tilman, 1978; Bell, 1982). If this mechanism can explain our data, asexual taxa should have been selected against at the lowest latitudes, and we would expect to see a clear difference in the lower range limit between sexual and PTH taxa. We did not find strong support for this pattern, as the average lower range limit of asexual species was only 2° higher than that of sexual species, and this difference was not significant (see Results; $P = 0.23$). An alternative mechanism proposed by Lynch (1984) is that asexuality is associated with greater plasticity and resilience to extreme physical conditions, which he argues occurs at higher latitudes. If asexual taxa are more phenotypically plastic, they should persist in a greater range of environments than do sexual taxa. Consequently, we might expect their limits to extend over latitudes that are both lower and higher than sexual taxa. As noted before, there was no clear pattern at lower latitudinal limits, but the upper range limits were 3.6° (*c.* 400 km) higher in asexual compared with sexual taxa, although this effect was not significant ($P = 0.13$). A final consideration is that asexual and sexual species are equally able to persist across all latitudes, but asexual taxa establish populations more quickly in high-latitude sites during range expansions, especially those that follow recent glaciation events (Bierzychudek, 1985; Law & Crespi, 2002). Given the observed upward shift in the median latitude of PTH species and a greater tendency for their upper range limits to extend beyond that of sexual taxa (compared with the lower range limits), we believe the observed patterns are most consistent with the third scenario brought about by differences in colonization following glaciation. This conclusion would be strengthened by additional experiments comparing the colonization ability and performance of PTH and sexual populations across latitudinal gradients within and outside of their current ranges.

We did not find strong support for the prediction that asexual taxa occupy larger range sizes. Bierzychudek's (1985) analysis of 41 species containing sexual and asexual lineages found larger ranges for asexual species in 76% of cases. However, as mentioned previously, asexuality and sexuality were confounded in this analysis, and a recent study of diploid and polyploid *Clarkia* spp. showed that ploidy can influence range size (Lowry & Lester, 2006). Moreover, recent comparisons of self-compatible and self-incompatible taxa have shown inconsistent and, at times, contradictory effects of selfing on range size (Lowry & Lester, 2006; Randle *et al.*, 2009).

The effects of selfing and asexuality on range size are likely to be more complicated than has been proposed. As pointed out by Stebbins (1950), the relationship between asexual reproduction and range size will probably depend on the age of different lineages. Asexual genotypes can have an ecological advantage over sexual genotypes which allows their populations to expand more rapidly and colonize new environments (Maynard Smith, 1978). However, asexual genotypes can accumulate deleterious mutations more readily than do sexual genotypes (Muller, 1964; Paland & Lynch, 2006), and exhibit a reduced ability to adapt to changes in the environment (Hill & Robertson, 1966; Otto & Lenormand, 2002). Such evolutionary processes are likely to counteract the ecological advantages of asexual reproduction and cause an eventual decrease in the growth rate of populations. Therefore, it is difficult to make any a priori predictions about the association between sex and range size without accounting for the age and mutation load of asexual lineages.

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Plant trait evolution

Our results indicate that theoretical predictions relating to the effects of selfing on the evolution of allocation to structures related to pollinator attraction, male reproduction and investment in female fitness also predict the effects of functionally asexual reproduction (Fig. 3). For example, we observed significant reductions in attractive structures (petals and floral tubes) and the length of anthers. There was also evidence for increased investment in seed mass, consistent with a greater investment in female reproduction. Importantly, we did not observe any changes in traits related to growth and resource acquisition, which would only be expected to occur if asexuality leads to correlated evolutionary changes throughout the genome, perhaps as a result of an accumulation of deleterious mutations that influence the expression of a random set of many traits. Therefore, our results suggest that PTH species underwent adaptive evolution following a loss of recombination and segregation.

Given the apparent capacity for adaptive evolution in PTH species, one might ask: why do functionally asexual organisms maintain attractive structures at all? Although there is a reduction in the size of attractive structures, PTH species maintain showy flowers, floral tubes and the production of nectar and floral volatiles. Similarly, many apomictic species, such as dandelions (*Taraxacum*), blackberries (*Rubus*), and St John's Wort (*Hypericum*), also maintain large flowers. It is often thought that such structures are maintained because asexual species are evolutionarily stuck and have little capacity to reduce floral traits. This may provide a partial explanation in that, even though PTH species have evolved smaller flowers, they may still experience strong constraints on adaptive evolution compared with sexual populations (Stebbins, 1950; Johnson *et al.*, 2009). Thus, even if selection favored a reduction in attractive structures, PTH populations may not present sufficient variation on which selection could act. A nonexclusive explanation could be that there has been insufficient time for asexual populations to reach an optimum, which seems likely in the case of those PTH species that are reported to have arisen very recently (Dietrich *et al.*, 1997). A final possibility is that some forms of asexuality select for the maintenance of attractive structures and male function. Noirot *et al.* (1997) have shown theoretically that, even in the complete absence of recombination and segregation, there can be selection for increased investment in male function (flowers and pollen). This occurs when pollen is required to set seed in functionally asexual plant species, such as pseudogamous apomicts, and an increase in pollen production results in increased seed set via either self- or cross-pollination. This seems less likely in the case of PTH species, which typically undergo fertilization before the opening of lowers, but we find it noteworthy that asexuality is not always associated with a reduction in male function and attractive structures.

Is asexuality a 'blind alley'?

A complete breakdown in recombination and segregation commits organisms to a functionally asexual reproductive strategy, whether this occurs via apomixis, parthenogenesis or PTH. Despite potential ecological advantages (Maynard Smith, 1978; Agrawal, 2006), conventional wisdom is that a loss of sex is an 'evolutionary blind alley' (Stebbins, 1950), suffering from reduced evolutionary potential, reduced rates of speciation and premature extinction when compared with sexually reproducing species (Darlington, 1939; Stebbins, 1950; Rice, 2002; Schwander & Crespi, 2009). Surprisingly, this paradigm is built upon little empirical data (Rice, 2002; Agrawal, 2006; Paland & Lynch, 2006; Otto, 2009). In our own system, we find that a breakdown in sex is associated with decreased defenses against generalist insect herbivores (Johnson *et al.*, 2009). Thus, in a coevolutionary context, a loss of sex decreases the ability for populations to maintain or reach an equilibrium in defense against parasites. The evidence presented here suggests that asexual species can undergo adaptive evolution of investment in reproductive characters, but whether these populations have reached an optimum is not yet clear.

Even though a loss of sexual reproduction has probably resulted in reduced rates of adaptive evolution and increased mutation loads, evidence suggests that a breakdown of sex in *Oenothera* does not lead to inevitable extinction. Previous ancestral state reconstructions suggest that there are cases in which a reversion from PTH to sexual reproduction has occurred, providing an escape to Darlington's 'blind alley' (Johnson *et al.*, 2009). In theory, this can occur through rare hybridization events, because PTH species, like many apomictic plant species, retain some ability to cross-pollinate. Evidence in support of this comes from experiments in which forced hybridization restored at least partial recombination and segregation in the F_1 progeny (Cleland, 1972); hybridization involving PTH and sexual species is also reported from natural populations (Dietrich *et al.*, 1997). Finally, there is evidence to suggest that similar reversions from apomixis to sexual reproduction are more prevalent than previously appreciated (Asker & Jerling, 1992; Menken *et al.*, 1995; Whitton *et al.*, 2008). Thus, the evolution of asexual reproduction might not be a one-way street down a blind alley, but instead a narrow, slow-moving, two-way street with occasional exits. If true, the evolutionary consequences of asexual reproduction are less restrictive than previously thought.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Raw data used in phylogenetic regression analyses

Table S2 List of taxa included in the phylogeny and Genbank accession numbers for each gene

Table S3 Statistics associated with alternative models of evolution for sexual and permanent translocation heterozygosity (PTH) reproduction

Table S4 Summary statistics and best-fitting substitution models for individual and combined datasets for phylogenetic analysis of *Oenothera* section *Oenothera*

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