Supporting Information

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SI Text

Molecular Sequence Data and Phylogenetic Inference. PCR products were directly sequenced when possible with the primers used to isolate the gene. Sequencing was performed by using an ABI 3730xl DNA sequencer and Big-Dye protocols (Applied Biosystems). Indel allelic length variants were detected at *PgiC* for a small number of species. In these cases we cloned the PCR products by using the pGEM-T Easy Vector System (Promega) and sequenced 4–6 clones per product to identify the alleles. No individuals showed >2 alleles, consistent with previous results that show *PgiC* is present as a single copy within *Oenothera* (1). Multiple sequences from each species were initially included in phylogenetic analyses, but because alleles from the same species consistently clustered together, we randomly selected a single representative sequence for each species for the final dataset.

We assessed the combinability of the datasets by using 2 methods. First, we conducted incongruence length difference (ILD) tests (2) between all pairs of genes. These tests were conducted in PAUP 4.0b10 (3) using 500 replicate partitions, each subjected to heuristic searches with simple taxon addition, TBR branch swapping, and keeping no more than 500 trees per replicate. ILD tests revealed that 2 plastid regions (*trnL-trnF* and *rps16*) could be combined ($P = 0.08$) as could the nuclear datasets (ITS, ETS, and *PgiC*) (P 0.10). However, the ILD suggested conflict between the nuclear and plastid datasets ($P = 0.002$). To further examine this potential conflict, we conducted bootstrap analyses of the nuclear and plastid datasets and examined consensus trees from these analyses for evidence of hard incongruence, i.e., bootstrap support 70% for conflicting clades (4). Bootstrap analyses were conducted with 500 replicates and heuristic searches with 10 random taxon addition sequences, keeping 100 trees per replicate. We observed no instances of hard incongruence between plastid and nuclear datasets within clades that contained PTH and sexual species; thus, we chose to combine the datasets for final analyses.

Bayesian analyses of the combined dataset comprised 2 independent runs with 4 linked chains executed with MrBayes v. 3.1.2 (http://mrbayes.csit.fsu.edu/index.php). Chains proceeded for 5 million generations, sampling every 100 generations. Convergence was assessed by examining the diagnostics in the sump output and comparing consensus topologies and branch lengths from the independent runs. We conservatively discarded the first 20% of trees as burn-in, leaving 40,000 trees for subsequent analyses.

Experimental Details. Experimental species were selected to maximize the number of independent transitions between sexual and PTH reproduction from across the phylogeny. In selecting sexual plant species we favored species that are partially outcrossing. Although a comprehensive dataset of outcrossing rates does not exist for *Oenothera* and *Gayophytum*, experimental crosses show that of the 16 sexual plant species studied here, 8 are self-incompatible (SI), 5 are self-compatible (SC) and at least partially outcrossing, and 2 are polymorphic for SI and SC (http://botany.si.edu/onagracceae/index.cfm).

All experimental plants were started as seed that originated from single plant populations or were propagated from plants collected from a single site. Whenever possible, we used seeds bulk collected from 10 maternal plants growing in large natural populations (100 plants). In the lab experiment, seeds from all species were germinated simultaneously on moistened filter paper and then transplanted to 250-mL pots containing Farfard 4P Mix soil (Conrad Fafard) , supplemented with 4 Osmocote slow release fertilizer pellets (14:14:14, N/P/K; The Scotts Company). Plants were randomized within a single growth room at Duke University set at 24 °C and a 16:8-h light/dark cycle. Plants were grown for 9 weeks before assaying susceptibility.

In the field experiment, plants were germinated from seed in April 2008, grown for 3 weeks in the growth chamber as before, and transplanted into a single large field in May 2008, located in Durham, NC. Plants were planted directly into the soil with 1-m spacing between rows and columns and watered periodically to prevent mortality caused by drought. To account for the effects of natural environmental variation, we randomized plants into 4 contiguous spatial blocks that were each $\approx 8 \times 15$ m in size. Before phylogenetic regression analyses, we tested for the effect of spatial block on percentage of herbivory. When there were significant block effects, we saved the residuals and performed phylogenetic regression analyses on these residuals; these data were transformed back to the original units by adding the experiment-wide average herbivory to all residual values.

Susceptibility to the specialist beetle (*Alticus foenae*) was assayed by using tissue collected from plants in the field experiment. Larvae of this beetle are only known to feed on *Oenothera* spp., and the adults specialize on *Oenothera* spp. as well but are able to colonize other Eurosids (e.g., apple) when their primary host plant is in short supply. Adult beetles were collected with an aspirator from several species of *Oenothera* (*O. biennis, O. humifusa*, *O. laciniata*, *O. simulans*) growing in Fort Macon State Park (Atlantic Beach, NC) where a population outbreak had occurred. We assayed susceptibility in early June 2008 by excising a single leaf from each plant grown in the field experiment, which were used for no-choice Petri dish assays as before. Individual beetles were allowed to feed for 22 h before removal; the amount of leaf tissue consumed was measured as described.

We measured five plant traits from plants grown in growth chambers during the initial lab experiment. Leaf toughness (reps: 2–32 plants/species; mean = 22) was measured as the grams of force required to penetrate a leaf using a force gauge penetrometer (Type 516: Chatillon, Kew Gardens, New York). Tannin concentrations (reps: 2–17 plants/species; mean = 10) was determined according to Hagerman's radial diffusion method (5) using finely ground leaf tissue. Tannin concentration was quantified as millimeters of precipitate formed by 20 μL of a 10% extract (w/v) of soluble phenolics added to a 1% agarose gel containing 0.1% BSA. Percent leaf water content (reps: 1–32 plants/species; mean = 20) was estimated from 0.26-cm^2 leaf discs cut using a hole punch from the leaf away from the midvein. Specific leaf area (SLA) (reps: 1–32 plants/species; mean = 20) was calculated as 0.26 cm^2/dry mass (g) of each leaf disc; all measurements were taken on a 10^{-6}g microbalance (Mettler Toledo, Columbus, OH). Trichome density (reps: 2–9 plants/species; mean = 5) was measured as the average number of leaf hairs on the upper and lower surface of leaf discs.

Phylogenetic Regression Analyses. We used phylogenetic comparative methods that incorporate measurement error within species (i.e., standard error of a species' mean trait value) using the programs: MEUnivarPHYSIG (estimates of K*), MECorrPHYSIG (estimates of r_{phylo}) and MERegPHYSIG (effects of PTH/sex on susceptibility and traits) in MATLAB 7.7 (The Mathworks) as described by Ives and colleagues (6). Specifically, we used restricted maximum likelihood to estimate parameters while incorporating information about the phylogeny, mean variation in traits among species, and standard errors associated

with each mean trait value. These methods model trait evolution according to Brownian motion evolution or trait evolution that is independent of phylogeny. The statistical significance of parameter estimates were estimated by parametric bootstrapping, whereby REML estimates of parameters were used to simulate 2,000 new datasets, from which new estimates of parameters were derived from these simulations and the distribution of these values were used to calculate approximate *P* values. Based on our *a priori* predictions, we performed 1-tailed tests of significance when testing the effects of sex on herbivore susceptibility, and 2-tailed tests otherwise. All analyses utilized mean trait values, the standard error of mean values, and the variance-covariance matrix calculated from the single maximum likelihood phylogeny inferred by RAxML (7) ultrametricized using NPRS in TreeEdit (http://tree.bio.ed.ac.uk/software/treeedit). PTH and sexual reproduction were coded as 0/1 dummy variables, respectively, with 0 standard error. Estimates of K^* (8), where 1 corresponds to Brownian motion evolution of traits and 0 corresponds to trait evolution independent of phylogeny – and comparisons with models that relax the assumption of Brownian motion evolution, showed that Brownian evolution typically provided a good fit to the data.

1. Ford VS, Gottlieb LD (2007) Tribal relationships within Onagraceae inferred from *PgiC* sequences. *Syst Bot* 32:348–356.
2. Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics* 10:315–319.
3. Swofford DL (2002) PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods) Version 4.0 Beta (Sinauer, Sunderland, MA).
4. Mason-Gamer RJ, Kellogg EA (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst Biol* 45:524–545.
5. Hagerman AE (1987) Radial diffusion method for determining tannin in plant extracts. *J Chem Ecol* 13:437–449.
6. Ives AR, Midford PE, Garland Jr. T (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Syst Biol* 56:252–270.
7. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
8. Blomberg SP, Garland Jr. T, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.

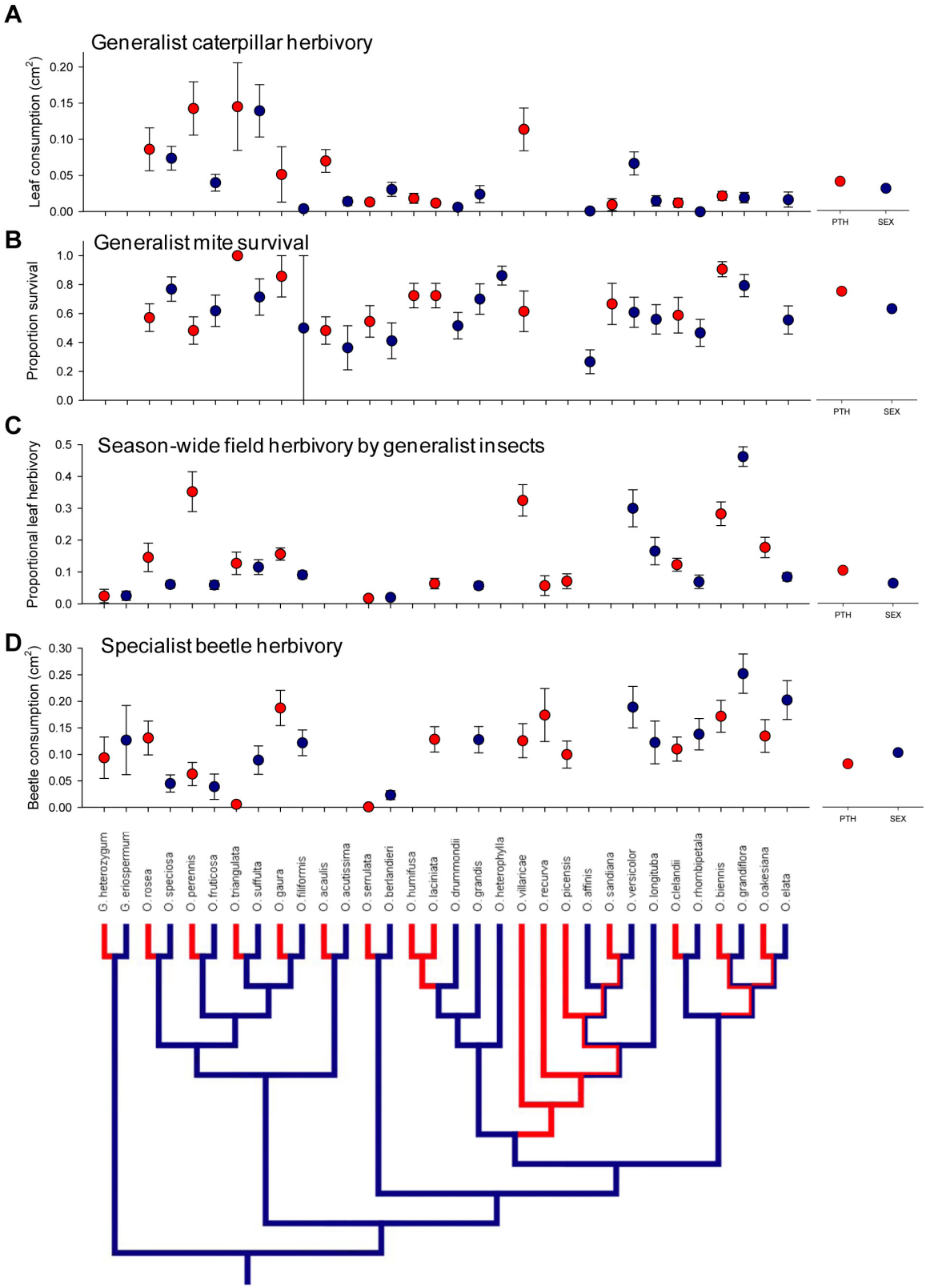

Fig. S1. Phylogenetic relationship of PTH and sexual plant species used in experiments. Four representative measures of susceptibility to arthropod herbivores are shown to illustrate variation among PTH (red) and sexual (blue) plant species for: consumption by the generalist caterpillar (*A*), proportional survival of mites (*B*), the proportion of herbivory on plants in the field (*C*), and consumption by the specialist beetle (*D*). Phylogenetically adjusted mean values are shown at the far right.

Table S1. Species of Onagraceae included in phylogenetic analyses

Species	Sex/PTH	*trnL-trnF*	*rps16*	*ITS*	*ETS*	*pgiC*
Fuchsia cyrtandroides (O)	Sex	AY264497	–	AY357779	–	*
Chaemerion angustifolium (O)	Sex	AY264505	AY267389	L28011	–	*
Epilobium canum (O)	Sex	AY264514	AY267391	L28012	–	–
Epilobium ciliatum (O)	Sex	AY264508	–	L28015	–	–
Gongylocarpus fruticolosus (O)	Sex	AY264504	AY267388	AY271527	–	–
Hauya elegans (O)	Sex	AY264496	–	AY357767	–	–
Lopezia racemosa (O)	Sex	AY264502	–	AY271525	–	–
Ludwigia Peploides (O)	Sex	AY264494	AY267386	AY271517	–	*
Camissonia campestris	Sex	AY264538	AY267413	AY271550	–	–
Camissonia kernensis	Sex	AY264537	AY267412	AY271549	–	–
Camissoniopsis cheiranthifolia	Sex	AY264536	AY267411	AY271548	–	–
Chylismia arenaria	Sex	AY264531	AY267406	AY271543	–	*
Chylismia claviformis	Sex	AY264529	AY267404	AY271541	–	–
Clarkia affinis	Sex	AY264524	AY267399	AY271536	–	–
Clarkia concinna	Sex	AY264519	–	AY271532	–	X89390
Clarkia delicata	Sex	AY264525	AY267400	AY271537	–	AJ311748
Clarkia franciscana	Sex	AY264517	AY267394	AY271530	–	X89396
Clarkia heterandra	Sex	AY264526	AY267401	AY271538	–	AJ437274
Clarkia imbricata	Sex	AY264520	AY267396	AY271533	–	–
Clarkia mildrediae	Sex	AY264516	AY267393	AY271529	–	X89389
Clarkia pulchella	Sex	AY264518	AY267395	AY271531	–	–
Clarkia rostrata	Sex	AY264523	AY267398	AY271535	–	X89392
Clarkia xantiana	Sex	AY264522	AY267397	AY271534	–	X80666
Eremothera boothii	Sex	AY264530	AY267405	AY271542	–	–
Eremothera minor	Sex	AY264541	AY267416	AY271553	–	–
Eremothera nevadensis	Sex	AY264540	AY267415	AY271552	–	–
Eremothera refracta	Sex	AY264539	AY267414	AY271551	–	–
Eulobus californicus	Sex	AY264585	AY267459	AY271597	–	–
Eulobus crassifolius	Sex	AY264528	AY267403	AY271540	–	–
Gayophytum d. parviflorum	Sex	*	–	*	–	–
Gayophytum diffusum diffusum	Sex	*	–	*	–	–
Gayophytum eriospermum†	Sex	*	–	*	–	*
Gayophytum heterozygum†	PTH	AY264527	AY267402	AY271539	–	–
Holmgrenia andina	Sex	AY264543	AY267418	AY271555	–	–
Oenothera acaulis†	PTH	*	–	*	–	*
Oenothera acutissima†	Sex	AY264563	–	AY271575	–	*
Oenothera affinis†	Sex	AY264551	AY267426	AY271563	–	*
Oenothera albicaulis	Sex	AY264554	AY267429	AJ620536	AJ620784	–
Oenothera anomala	Sex	AJ620597	AY267450	AJ620529	AJ620777	–
Oenothera argillicola	Sex	*	–	*	–	*
Oenothera arida	Sex	AJ620596	AY267452	AJ620528	AJ620775	–
Oenothera bahia-blancae	PTH	*	–	*	–	*
Oenothera berlandieri†	Sex	AY264574	AY267448	AY271586	–	*
Oenothera biennis†	PTH	AY264553	AY267428	DQ006021	–	*
Oenothera boquillensis	Sex	AJ620587	AY267453	AJ620518	AJ620765	–
Oenothera brachycarpa	Sex	AY264560	AY267435	AY271572	–	–
Oenothera caespitosa	Sex	AY264558	AY267433	AY271570	–	–
Oenothera canescens	Sex	AY264564	AY267438	AY271576	–	–
Oenothera cinerea	Sex	AJ620601	AY267458	AJ620535	AJ620782	–
Oenothera clelandii†	PTH	*	–	*	–	*
Oenothera curtiflora	Sex	AJ620584	AY267451	AJ620515	AJ620762	–
Oenothera deltoides	Sex	AY264572	AY267446	DQ075620	–	–
Oenothera demareei	Sex	AJ620590	AY267455	AJ620522	AJ620769	–
Oenothera drummondii†	Sex	AJ620605	–	AJ620537	AJ620785	*
Oenothera elata hookeri†	Sex	AY264552	AY267427	DQ075625	–	*
Oenothera elongata	PTH	*	–	*	–	*
Oenothera filiformis†	Sex	AJ620595	–	AJ620527	AJ620774	–
Oenothera filipes	Sex	AJ620592	–	AJ620524	AJ620771	–
Oenothera flava	Sex	AY264562	AY267437	DQ075624	–	–
Oenothera fruticosa†	Sex	AY264569	AY267443	AY271581	–	–
Oenothera gaura†	PTH	AJ620586	–	AJ620517	AJ620764	*
Oenothera glaucifolia	Sex	AJ620602	AY267449	AJ620542	AJ620783	–
Oenothera glazioviana	PTH	*	–	*	–	*
Oenothera grandiflora†	Sex	*	–	*	–	*

Species	Sex/PTH	*trnL-trnF*	*rps16*	*ITS*	*ETS*	*pgiC*
Oenothera grandis†	Sex	*	–	*	–	*
Oenothera hartwegii	Sex	AY264573	AY267447	AY271585	–	–
Oenothera havardii	Sex	AY264561	AY267436	AY271573	–	–
Oenothera heterophylla†	Sex	AY264548	AY267423	AJ620538	AJ620786	*
Oenothera hexandra	Sex	AJ620593	AY267456	AJ620525	AJ620772	–
Oenothera humifusa†	PTH	*	–	*	–	*
Oenothera kunthiana	PTH	*	–	*	–	*
Oenothera laciniata†	PTH	AJ620607	AY267424	AJ620539	AJ620787	*
Oenothera lavandulifolia	Sex	AJ620603	–	AJ620543	AJ620790	–
Oenothera lindheimeri	Sex	AJ620594	–	AJ620526	AJ620773	AJ550748
Oenothera linifolia	Sex	AY264586	AY267460	AY271598	–	–
Oenothera longissima	Sex	*	–	*	–	*
Oenothera longituba†	Sex	*	–	*	–	*
Oenothera macrocarpa	Sex	AJ620608	–	AJ620540	AJ620788	–
Oenothera macrosceles	Sex	AY264546	AY267421	AY271558	–	–
Oenothera maysillesii	Sex	AY264545	AY267420	AY271557	–	–
Oenothera mendocinensis	Sex	*	–	*	–	*
Oenothera multicaulis	PTH	AY264568	AY267442	AY271580	–	–
Oenothera nana	PTH	*	–	*	–	*
Oenothera neomexicana	Sex	AY264570	AY267444	DQ075628	–	–
Oenothera nutans	PTH	*	–	*	–	*
Oenothera oakesiana†	PTH	*	–	*	–	*
Oenothera organensis	Sex	AY264544	AY267419	DQ075630	–	–
Oenothera pallida	Sex	AY264571	AY267445	DQ075632	–	–
Oenothera paradonia	PTH	*	–	*	–	*
Oenothera patriciae	Sex	AJ620588	–	AJ620519	AJ620766	–
Oenothera perennis†	PTH	*	–	*	–	*
Oenothera picensis picensis†	PTH	*	–	*	–	*
Oenothera primiveris	Sex	AY264556	AY267431	DQ075635	–	–
Oenothera psammophila	Sex	AY264559	AY267434	AY271571	–	–
Oenothera pubescens	PTH	AY264550	AY267425	AY271562	–	–
Oenothera ravenii chilensis	PTH	*	–	*	–	*
Oenothera recurva†	PTH	*	–	*	–	*
Oenothera rhombipetala	Sex	*	–	*	–	*
Oenothera rosea	PTH	AY264566	AY267440	AY271578	–	–
Oenothera sandiana†	PTH	*	–	*	–	*
Oenothera serrulata†	PTH	*	–	*	–	*
Oenothera simulans	Sex	AJ620585	–	AJ620516	AJ620763	–
Oenothera sinuosa	Sex	AJ620598	–	AJ620532	AJ620779	–
Oenothera speciosa†	Sex	AJ620609	AY267439	AJ620541	AJ620789	–
Oenothera stricta stricta	PTH	*	–	*	–	*
Oenothera stubbei	Sex	AY264547	AY267422	DQ075637	–	–
Oenothera suffrutescens	Sex	AJ620589	–	AJ620521	AJ620768	–
Oenothera suffulta†	Sex	AJ620599	–	AJ620533	AJ620780	–
Oenothera tarijensis	PTH	*	–	*	–	*
Oenothera tetraptera	Sex	AY264567	AY267441	DQ075638	–	–
Oenothera toumeyi	Sex	*	–	*	–	AJ550747
Oenothera triangulata†	PTH	AJ620600	–	AJ620534	AJ620781	–
Oenothera tubifera	Sex	AY264555	AY267430	DQ075639	–	–
Oenothera versicolor†	Sex	*	–	*	–	*
Oenothera villaricae†	PTH	*	–	*	–	*
Oenothera villosa†	PTH	*	–	*	–	*
Oenothera xylocarpa	Sex	AY264557	AY267432	DQ075641	–	–
Taraxia ovata	Sex	AY264532	AY267407	AY271544	–	–
Taraxia tanacetifolia	Sex	AY264534	AY267409	AY271546	–	–
Tetrapteron graciliflorum	Sex	AY264535	AY267410	AY271547	–	–
Xylonagra arborea	Sex	AY264515	AY267392	AY271528	–	–

Our sampling included 113 species in the Onagreae tribe and 8 additional outgroup (O) species from the Onagraceae. We indicate the reproductive system of species according to whether a species predominantly uses a PTH genetic system or exhibits functional recombination and segregation among 2 or more pairs of chromosomes (Sex). For each gene we indicate the source of the data, according to Genbank accession numbers, species newly sequenced in this study (*), or whether data were obtained (–). Species used in experiments are denoted by †.

Table S2. Effects of PTH and sexual reproduction on susceptibility to herbivores and plant traits

Variable	PTH	Sex	*P value*	% effect size	K* (*P* value)	Sex-PTH
Generalist caterpillar						
Consumption, cm^2	0.041	0.031	<0.01	31.6	$0.63^{n.s.}$	−0.010
Weight gain, mg	0.126	0.112	0.05	12.9	$0.92^{n.s.}$	−0.014
Proportion survival	0.452	0.375	<0.01	20.6	$0.59^{n.s.}$	−0.077
Generalist mite						
Proportion survival	0.678	0.568	<0.01	19.3	$0.58^{n.s.}$	−0.110
No. of eggs	0.217	0.199	>0.1	9.3	$0.77^{n.s.}$	−0.018
Specialist beetle						
Consumption, cm^2	0.080	0.101	<0.01	−20.9	$0.67^{n.s.}$	0.021
Proportion herbivory in the field						
Seasonwide herbivory	0.103	0.063	<0.01	64.0	0.30*	−0.040
Proportion herbivory, May	0.007	0.007	>0.05	−3.4	$1^{n.s.}$	0.000
Proportion herbivory, June	0.054	0.044	<0.01	22.8	$0.55^{n.s.}$	−0.010
Proportion herbivory, July	0.072	0.039	<0.01	83.4	0.32*	−0.033
Proportion herbivory, August	0.061	0.054	0.04	12.6	0.36*	−0.007
Plant traits						
Leaf toughness, g of force	86.15	143.97	<0.01	−39.6	0.22*	57.81
Tannins, mm	1.99	2.22	<0.01	−11.2	0.42*	0.22
Trichomes, no./cm^2	84.31	64.00	<0.01	21.2	0.21*	−20.31
% water content	78.08	77.88	>0.05	0.9	$0.65^{n.s.}$	−0.20
Specific leaf area, cm^2/g	202.26	190.89	<0.01	5.3	0.46*	−11.36

For each variable we show phylogenetically adjusted mean values for PTH and sexual species, estimated by restricted maximum likelihood in MERegPHYSIG as described by Ives et al. (1). We show *P* values estimated by parametric bootstrapping to assess the statistical significance of the difference between mean values. Effect sizes indicate the percentage of increase/decrease in susceptibility on PTH species relative to sexual plant species. The phylogenetic signal in each trait was estimated according to K* (2); an estimate of 1 indicates signal consistent with Brownian motion evolution and 0 indicates no phylogenetic signal. We use * to indicate K* values that were significantly different from 1 and not signifcant (n.s.) for values that did not significantly deviate from 1.

1. Ives AR, Midford PE, Garland T, Jr (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Syst Biol* 56:252–270.
2. Blomberg SP, Garland T, Jr, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.

Table S3. Correlations in the susceptibility of herbivores when feeding on *Oenothera* and *Gayophytum* species

Variable 1	Variable 2	r_{phylo}	L 95 CI	U 95 CI
Cat* consumption	Cat weight	0.70	0.34	0.90
Cat consumption	Cat survival	0.72	0.40	0.92
Cat consumption	Mite survival	0.67	0.20	0.98
Cat consumption	Mite eggs	−0.52	−0.92	0.06
Cat consumption	Field herbivory†	0.73	0.32	0.94
Cat consumption	Beetle consumption	−0.68	−0.99	−0.16
Cat weight	Cat survival	0.55	0.09	0.85
Cat weight	Mite survival	0.48	−0.15	0.93
Cat weight	Mite eggs	−0.27	−0.86	0.43
Cat weight	Field herbivory	0.57	−0.10	0.93
Cat weight	Beetle consumption	−0.50	−1.00	0.24
Cat survival	Mite survival	0.74	0.34	0.98
Cat survival	Mite eggs	−0.66	−0.97	−0.18
Cat survival	Field herbivory	0.74	0.34	0.95
Cat survival	Beetle consumption	−0.74	−0.99	−0.28
Mite survival	Mite eggs	−0.66	−0.99	−0.17
Mite survival	Field herbivory	0.73	0.18	1.00
Mite survival	Beetle consumption	−0.74	−1.00	−0.19
Mite eggs	Field herbivory	−0.64	−1.00	0.02
Mite eggs	Beetle consumption	0.71	−0.04	1.00
Field herbivory	Beetle consumption	−0.69	−0.94	−0.32

Correlation coffecients (r_{phylo}) were estimated by REML in MECorrPhysig of Ives et al. (1) assuming a Brownian motion model of trait evolution across the phylogeny. Lower (L) and upper (U) 95% confidence intervals (CI) were estimated by parametric bootstrapping.
*Cat refers to beet armyworm caterpillar *Spodoptera exigua*.
†Seasonwide maximum herbivory measured on individual plants.

1. Ives AR, Midford PE, Garland T, Jr (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Syst Biol* 56:252–270.

Table S4. Correlations between herbivore susceptibility and the traits of *Oenothera* and *Gayophytum* spp. used in experiments

Variable 1	Variable 2	r_{phylo}	L 95 CI	L 95 CI
Cat*consumption	Leaf toughness	**−0.67**	**−0.90**	**−0.28**
Cat consumption	Tannin concentration	**−0.69**	**−0.91**	**−0.32**
Cat consumption	Trichome density	0.29	−0.21	0.71
Cat consumption	% leaf water content	**0.66**	**0.25**	**0.93**
Cat consumption	Specific leaf area	**0.55**	**0.03**	**0.89**
Cat weight	Leaf toughness	−0.46	−0.84	0.06
Cat weight	Tannin concentration	−0.51	−0.85	0.02
Cat weight	Trichome density	0.10	−0.45	0.62
Cat weight	% leaf water content	0.53	−0.04	0.88
Cat weight	Specific leaf area	0.46	−0.12	0.87
Cat survival	Leaf toughness	**−0.74**	**−0.93**	**−0.46**
Cat survival	Tannin concentration	**−0.74**	**−0.93**	**−0.43**
Cat survival	Trichome density	0.41	−0.03	0.75
Cat survival	% leaf water content	**0.72**	**0.39**	**0.92**
Cat survival	Specific leaf area	**0.55**	**0.14**	**0.84**
Mite survival	Leaf toughness	**−0.75**	**−0.40**	**−0.97**
Mite survival	Tannin concentration	**−0.73**	**−0.97**	**−0.37**
Mite survival	Trichome density	**0.50**	**0.01**	**0.85**
Mite survival	% leaf water content	**0.71**	**0.31**	**0.95**
Mite survival	Specific leaf area	**0.55**	**0.12**	**0.90**
Mite eggs	Leaf toughness	**0.69**	**0.14**	**1.00**
Mite eggs	Tannin concentration	**0.66**	**0.07**	**1.00**
Mite eggs	Trichome density	−0.44	−1.00	0.29
Mite eggs	% leaf water content	−0.71	−1.00	0.67
Mite eggs	Specific leaf area	**−0.55**	**−0.54**	**−0.42**
Field herbivory	Leaf toughness	**−0.74**	**−0.93**	**−0.41**
Field herbivory	Tannin concentration	**−0.72**	**−0.93**	**−0.39**
Field herbivory	Trichome density	**0.70**	**0.30**	**0.93**
Field herbivory	% leaf water content	**0.67**	**0.26**	**0.91**
Field herbivory	Specific leaf area	**0.59**	**0.14**	**0.90**
Beetle consumption	Leaf toughness	**0.75**	**0.37**	**0.96**
Beetle consumption	Tannin concentration	**0.74**	**0.34**	**0.98**
Beetle consumption	Trichome density	**−0.66**	**−0.95**	**−0.18**
Beetle consumption	% leaf water content	**−0.74**	**−0.97**	**−0.33**
Beetle consumption	Specific leaf area	**−0.61**	**−0.93**	**−0.09**

Correlation coffecients (r_{phylo}) were estimated by REML in MECorrPhysig assuming a Brownian motion model of trait evolution across the phylogeny. Lower (L) and upper (U) 95 confidence intervals (CI) were estimated by parametric bootstrapping. Correlation coefficients where $P < 0.05$ (based on CI) are shown in bold.

*Cat refers to beet armyworm caterpillar *Spodoptera exigua*.

Table S5. Primers used in this study

Gene	Primer name	Primer sequence	Ref./source
trnL-trnF	c, d, e, f		1
ITS	AB101, AB102		2, 3
PgiC	pgiC_oeno_F1	TTC GCC AGC TTT GAT CAG C	Designed for this study
	pgiC_oeno_F2i	TGA CAC AAT TAA TAA GCT C	Designed for this study
	pgiC_oeno_R2i	AAC CAC ATT CTT TCC ATC G	Designed for this study
	pgiC_oeno_R3	ACG GCC TCT AGC ATT CTT AC	Designed for this study
	pgiC_oeno_F1b	GAA GGA TTT GAA GGT A	Designed for this study
	pgiC_oeno_F3ib	CCT GGC AGA TAA ACA GTA CA	Designed for this study
	pgiC_oeno_R2ib	TGT ACT GTT TAT CTG CCA G	Designed for this study
	pgiC_oeno_F3b	AMA CTT ACA AAC GCA G	Designed for this study

Primer sequences are written 5′ to 3′ and are given only when designed specifically for this study. Primers labeled with the letter ''i'' denote internal primers. Primers labeled with the letter ''b'' denote primers designed to amplify *PgiC* from *Fuchsia* and *Ludwigia*.

1. Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Mol Biol* 17:1105–1109.
2. Douzery EJP, *et al.* (1999) Molecular phylogenetics of Diseae (Orchidaceae): A contribution from nuclear ribosomal ITS sequences. *Am J Bot* 86:887–899.
3. Hoggard GD, Kores PJ, Molvray M, Hoggard RK (2004) The phylogeny of *Gaura* (Onagraceae) based on ITS, ETS, and *TrnL-F* sequence data. *Am J Bot* 91:139–148.

Table S6. Herbivore susceptibility data used in phylogenetic regression analyses

		Generalist caterpillar						Generalist mite							
		Consumption		Wet weight		Survival		Survival		No. eggs		Field herbivory, %		Specialist beetle consumption	
Species	PTH/Sex	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
G. heterozygum	PTH	—	—	—	—	—	—	—	—	—	—	0.025	0.021	0.094	0.039
G. eriospermum	Sex	—	—	—	—	—	—	—	—	—	—	0.025	0.015	0.127	0.065
O. speciosa	Sex	0.074	0.016	0.265	0.041	0.867	0.063	0.769	0.084	0.607	0.163	0.061	0.012	0.045	0.016
O. rosea	PTH	0.086	0.030	0.230	0.058	0.429	0.095	0.571	0.095	0.179	0.090	0.146	0.045	0.131	0.032
O. fruticosa	Sex	0.040	0.012	0.143	0.054	0.364	0.105	0.619	0.109	0.250	0.092	0.059	0.014	0.039	0.024
O. perennis	PTH	0.143	0.037	0.611	0.192	0.552	0.094	0.483	0.094	0.567	0.218	0.352	0.062	0.063	0.022
O. triangulata	PTH	0.145	0.060	1.066	0.370	0.875	0.125	1.000	0.000	0.778	0.434	0.127	0.035	0.006	0.003
O. suffulta	Sex	0.139	0.036	0.791	0.216	0.813	0.101	0.714	0.125	1.978	0.643	0.115	0.023	0.089	0.027
O. gaura	PTH	0.051	0.038	0.219	0.151	0.286	0.184	0.857	0.143	1.429	0.948	0.156	0.020	0.187	0.033
O. filiformis	Sex	0.004	0.004	0.035	0.005	0.500	0.500	0.500	0.500	0.000	0.000	0.091	0.012	0.122	0.024
O. acaulis	PTH	0.070	0.016	0.430	0.118	0.567	0.092	0.483	0.094	0.425	0.160	—	—	—	—
O. acutissima	Sex	0.014	0.006	0.054	0.013	0.200	0.133	0.364	0.152	0.091	0.091	—	—	—	—
O. serrulata	PTH	0.013	0.004	0.115	0.061	0.250	0.090	0.545	0.109	0.159	0.076	0.017	0.007	0.001	0.001
O. berlandieri	Sex	0.031	0.010	0.116	0.033	0.364	0.105	0.412	0.123	0.111	0.076	0.020	0.006	0.023	0.008
O. heterophylla	Sex	—	—	0.059	0.020	0.138	0.065	0.862	0.065	0.379	0.126	—	—	—	—
O. grandis	Sex	0.024	0.012	0.119	0.050	0.190	0.088	0.700	0.105	0.286	0.122	0.057	0.011	0.128	0.025
O. drummondii	Sex	0.006	0.002	0.049	0.009	0.194	0.072	0.516	0.091	0.161	0.067	—	—	—	—
O. humifusa	PTH	0.018	0.007	0.089	0.028	0.241	0.081	0.724	0.084	0.207	0.077	—	—	—	—
O. laciniata	PTH	0.012	0.004	0.047	0.008	0.172	0.071	0.724	0.084	0.414	0.145	0.064	0.016	0.128	0.024
O. villaricae	PTH	0.114	0.029	0.436	0.163	0.667	0.126	0.615	0.140	0.250	0.155	0.325	0.049	0.126	0.032
O. affinis	Sex	0.001	0.001	0.030	0.000	0.000	0.000	0.267	0.082	0.133	0.063	—	—	—	—
O. picensis	PTH	—	—	—	—	—	—	—	—	—	—	0.071	0.023	0.100	0.025
O. sandiana	PTH	0.010	0.008	0.034	0.004	0.077	0.077	0.667	0.142	0.154	0.104	—	—	—	—
O. versicolor	Sex	0.067	0.016	0.178	0.044	0.565	0.106	0.609	0.104	0.348	0.119	0.300	0.058	0.189	0.039
O. longituba	Sex	0.015	0.007	0.058	0.015	0.125	0.069	0.560	0.101	0.200	0.082	0.166	0.043	0.122	0.040
O. recurva	PTH	—	—	—	—	—	—	—	—	—	—	0.057	0.031	0.174	0.050
O. clelandii	PTH	0.012	0.006	0.054	0.016	0.167	0.090	0.588	0.123	0.111	0.076	0.123	0.020	0.110	0.023
O. rhombipetala	Sex	0.000	0.000	0.032	0.002	0.000	0.000	0.467	0.093	0.233	0.092	0.069	0.021	0.138	0.030
O. biennis	PTH	0.022	0.006	0.097	0.018	0.355	0.087	0.906	0.052	0.875	0.276	0.283	0.037	0.172	0.030
O. grandiflora	Sex	0.019	0.007	0.098	0.026	0.276	0.084	0.793	0.077	0.483	0.146	0.463	0.031	0.252	0.037
O. oakesiana	PTH	—	—	—	—	—	—	—	—	—	—	0.177	0.032	0.135	0.031
O. elata	Sex	0.017	0.010	0.123	0.064	0.148	0.070	0.556	0.097	0.407	0.228	0.085	0.013	0.202	0.037

1. Wagner WL, Hoch PC, Raven PH (2007) Revised classification of the Onagraceae. *Syst Bot Monog* 83:1–222.

Table S7. Plant trait data used in phylogenetic regression and correlation analyses

	Leaf toughness		Tannin concentration		Trichome density		% water		SLA	
Species	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
O. speciosa	108.13	7.91	1.82	0.18	129.71	26.58	76.11	1.29	183.58	10.59
O. rosea	62.84	4.16	2.33	0.31	81.40	14.90	69.74	1.41	217.97	10.80
O. fruticosa	100.45	6.58	2.78	0.23	0.60	0.40	80.71	0.81	240.51	15.76
O. perennis	66.83	5.54	2.85	0.30	2.42	1.20	77.42	1.05	259.29	12.10
O. triangulata	84.29	23.10	0.67	0.14	28.10	7.53	83.67	1.10	216.31	11.98
O. suffulta	129.67	10.64	0.82	0.10	114.10	13.85	77.52	1.27	199.10	13.10
O. gaura	102.86	11.27	2.77	0.23	39.70	6.49	68.58	3.39	159.61	18.57
O. filiformis	128.75	21.25	3.81	0.06	34.25	10.75	-	-	-	-
O. acaulis	106.67	8.63	1.19	0.15	82.92	23.71	78.92	0.97	168.20	6.99
O. acutissima	215.45	15.07	1.75	0.18	33.60	7.75	77.50	1.67	156.45	24.23
O. serrulata	100.37	5.87	2.76	0.44	58.79	11.01	83.27	0.85	218.90	8.82
O. berlandieri	98.27	8.34	1.88	0.43	49.40	16.32	83.45	0.77	240.55	17.48
O. heterophylla	107.14	7.70	1.39	0.26	85.61	13.40	78.55	1.07	203.62	15.60
O. grandis	67.05	7.26	1.48	0.46	82.60	7.78	85.84	0.98	320.21	21.88
O. drummondii	102.66	3.99	1.54	0.20	203.10	24.73	85.67	0.59	175.18	7.41
O. humifusa	84.91	6.02	1.63	0.13	163.20	15.99	83.99	0.78	194.00	9.06
O. laciniata	110.43	6.04	1.54	0.24	68.83	9.77	82.77	0.99	219.69	12.82
O. villaricae	149.17	13.30	2.44	0.18	51.00	13.27	79.89	1.17	206.32	15.06
O. affinis	113.17	9.05	1.82	0.14	164.30	29.43	76.10	0.92	191.27	8.02
O. sandiana	86.79	7.48	2.88	0.31	445.60	33.47	78.29	0.67	231.55	12.71
O. versicolor	172.21	10.25	2.54	0.21	155.70	41.96	79.91	0.68	193.85	8.74
O. longituba	301.20	12.70	3.63	0.26	13.40	5.28	74.64	0.62	140.75	3.93
O. clelandii	112.50	9.28	2.57	0.23	86.50	13.60	76.01	1.63	158.10	7.35
O. rhombipetala	186.52	9.82	3.09	0.15	72.10	18.10	74.72	1.25	153.04	14.40
O. biennis	140.00	8.09	2.78	0.25	113.75	21.35	71.02	0.85	150.22	6.45
O. grandiflora	152.58	9.42	3.56	0.23	177.80	17.34	67.89	0.76	129.58	3.89
O. elata	131.85	8.86	2.25	0.21	176.80	38.48	74.29	1.12	158.09	13.83

We show the means and standard errors for all plant traits measured from *Oenothera* species. The methods used in collecting data and the units of each variable are provided in *SI Text*. The reproductive system used by each species is given in Fig. 1 and Table S1.