Genetic Diversity and the Evolutionary History of Plant Immunity Genes in Two Species of *Zea*

David A. Moeller and Peter Tiffin

Department of Plant Biology, University of Minnesota, Saint Paul

Plant pathogenesis-related genes (PR genes) code for enzymes, enzyme inhibitors, and other peptides that confer resistance to pathogens and herbivores. Although several PR genes have been the subject of molecular population genetic analyses, a general understanding of their long-term evolutionary dynamics remains incomplete. Here we analyze sequence data from 17 PR genes from two closely related teosinte species of central Mexico. In addition to testing whether patterns of diversity at individual loci depart from expectations under a neutral model, we compared patterns of diversity at defense genes, as a class, to nondefense genes. In *Zea diploperennis*, the majority of defense genes have patterns of diversity consistent with neutral expectations while at least two genes showed evidence of recent positive selection consistent with arms-race models of antagonistic coevolution. In *Zea mays* ssp. *parviglumis*, by contrast, analyses of both defense and nondefense genes revealed strong and consistent departures from the neutral model, suggestive of nonequilibrium population dynamics or population structure. Nevertheless, we found a significant excess of replacement polymorphism in defense genes compared to nondefense genes. Although we cannot exclude relaxed selective constraint as an explanation, our results are consistent with temporally variable (transient or episodic) selection or geographically variable selection acting on *parviglumis* defense genes. The different patterns of diversity found in the two *Zea* species may be explained by *parviglumis*' greater distribution and population structure together with geographic variation in selection.

Introduction

Plant pathogenesis–related (PR) proteins constitute an integral part of the plant immune system (Nimchuk et al. 2003). Specifically, the PR proteins, as a class, are induced following plant recognition of herbivore or pathogen attack and directly inhibit parasite growth, reproduction, or infection (Ward et al. 1991; Morris et al. 1998; Muthukrishnan et al. 2001). In view of evidence that PR proteins play a central role in defense and that plant enemies impose selection on resistance traits in natural populations (Rausher 2001; de Meaux and Mitchell-Olds 2003), the genes that encode these proteins are likely candidates for evolving in response to selection. Unlike plant R genes which are involved in pathogen recognition and have consistently shown evidence for strong selection (Meyers et al. 1998; Wang et al. 1998; Caicedo, Schaal, and Kunkel 1999; Stahl et al. 1999; Bergelson et al. 2001; Mondragon-Palomino et al. 2002; Tian et al. 2002; Mauricio et al. 2003; Rose et al. 2004), molecular population genetic evidence for selection acting on PR genes is variable. Some PR genes show evidence for having evolved in response to strong positive selection. For example, positive selection appears to have driven among-species divergence in chitinase genes in *Arabis* (Bishop, Dean, and Mitchell-Olds 2000), β-1-3-endoglucanases in *Glycine* species (Bishop et al. 2005), and polygalacturonases among dicot species (Stotz et al. 2000). Patterns of intraspecific diversity suggest that positive selection has acted on at least one chitinase gene in *Zea* (Tiffin 2004) and a protease inhibitor in *Arabidopsis thaliana* (Clauss and Mitchell-Olds 2003). Many PR genes, however, show patterns of intraspecific diversity that are consistent with neutral expectations (Kawabe and Miyashita 1999; Tiffin and Gaut 2001*a*; Clauss and Mitchell-Olds 2003; Tiffin 2004).

Key words: coevolution, disease resistance, innate immunity, inducible defense, natural selection, population structure.

E-mail: ptiffin@umn.edu.

The apparently neutral evolutionary histories of several PR genes may indicate that these genes evolve under strong functional constraints or that their selective histories are not easily detected using standard molecular analyses. For example, temporally or spatially variable selection may result in patterns of nucleotide polymorphism that do not conform to expectations under simple models of balancing or positive selection (de Meaux and Mitchell-Olds 2003). For many plant species, signatures of selection may also be obscured by nonequilibrium population dynamics or population structure (Przeworski 2002; B. Charlesworth, D. Charlesworth, and Barton 2003; Wright and Gaut 2005). An additional problem for evaluating the evolutionary history of PR genes is that evidence of selection may not be detected due to low statistical power associated with the relatively short gene lengths and small number of segregating sites found in many PR genes. Combining data from multiple PR genes and comparing the levels and patterns of diversity to a set of reference genes that play no role in host defense may increase the statistical power for detecting selection and improve the ability to distinguish the effects of selection from demographic history.

The potential power of combining data from multiple genes in order to understand the evolutionary history of host defense has been demonstrated by two recent studies of innate immunity genes in *Drosophila*. Schlenke and Begun (2003) compared patterns of diversity at 34 *Drosophila simulans* immunity genes primarily involved in pathogen recognition and signaling to a collection of nonimmunity genes. Their analyses revealed higher values of Ka/Ks and lower haplotype diversity for immunity genes, patterns consistent with predictions of recurrent positive selection from arms-race models of coevolution (Van Valen 1973; Dawkins and Krebs 1979). By contrast, Lazzaro and Clark (2003) examined nine inducible antibacterial peptide genes from *Drosophila melanogaster* and found evidence for an excess of polymorphic replacement sites, a pattern of diversity potentially suggesting transient positive selection

favoring rare amino acid variants. Not all combined analyses of immunity-related genes, however, have detected evidence for nonneutral evolution. An analysis of seven peptidoglycan recognition genes thought to be involved in parasite recognition in *D. melanogaster* revealed no evidence for directional or balancing selection (Jiggins and Hurst 2003). Broad surveys of immunity genes coupled with comparisons of immunity to nonimmunity genes have not been conducted previously for plants.

Here we report on a survey of sequence variation in 17 pathogenesis-related genes (hereafter referred to as defense genes) in two closely related teosinte species, *Zea diploperennis* and *Zea mays* ssp. *parviglumis*. Our survey includes genes coding for eight types of PR proteins (see Supplementary Material Table 3 for further information), most of which are induced following plant perception of pathogen infection and/or herbivore attack: three chitinases, one β-1-3-glucanase, three protease inhibitors, one cysteine protease, three thaumatin-like proteins, two phospholipid transferases, two ribosomal-inactivating proteins, and two PR-1 proteins. Each of the genes we examine has been implicated as being functionally important in defending *Zea* against potential enemies or exhibits high similarity to genes that have been identified as functionally important in other species. Chitinases and β-1-3-glucanases degrade the chitin and glucan matrix of fungal cell walls, respectively (Schlumbaum et al. 1986; Huynh et al. 1992), the cysteine protease, *mir1*, damages the peritrophic matrix of the insect gut (Pechan et al. 2002), protease inhibitors inactivate digestive proteases in insect guts and proteases secreted by pathogens into host tissue (Ryan 1990), thaumatin-like proteins retard the growth of fungal pathogens and some may inhibit amylases and trypsins in herbivores (Velazhahan, Datta, and Muthukrishnan 1999), lipid transfer proteins exhibit antibiotic activity possibly by perforating pathogen plasma membranes (Kader 1996), ribosome-inactivating proteins disrupt protein synthesis in fungal pathogens by damaging ribosomes (Jensen et al. 1999; Nielsen, Payne, and Boston 2001), and PR-1 proteins are expressed in response to fungal and viral infection and some (e.g., *prms*) appear to be targeted to plasmodesmata to localize and prevent the spread of infection (Murillo, Cavallarin, and San Segundo 1997; Buchel and Linthorst 1999). Because plant PR genes are induced following damage and function by directly inhibiting pathogen infection, these genes are functionally similar to the effector proteins involved in innate immune response in insects and vertebrates (Menezes and Jared 2002).

The specific objectives of this study were twofold. First, we tested for evidence of selection acting on individual genes using analyses based on the frequency spectrum of intraspecific polymorphisms. Second, in order to determine if defense genes, when treated as a functional class, differ from nondefense genes in their evolutionary dynamics, we conducted several multilocus tests that compared patterns of polymorphism and divergence at defense genes to patterns at nondefense genes. Comparing the results of multilocus tests with single-locus tests allowed us to evaluate the extent and nature of genome-wide demographic effects that may shape diversity and obscure evidence of selection.

Materials and Methods
Species Sampling and Sequence Collection

We analyzed sequence data from 16 defense and 4 nondefense genes from *Z. diploperennis* (data for 12 of the defense genes are new to this study) and 17 defense and 10 nondefense genes from *Z. mays* ssp. *parviglumis* (hereafter *parviglumis*) (data for 13 of the defense genes are new to this study). Both species are wind-pollinated endemics of southwestern and south-central Mexico. *Zea diploperennis* is a perennial that is restricted to a small, high-elevation region of Sierra de Manantlán in southwestern Jalisco (Iltis et al. 1979), while *parviglumis*, the closest relative and progenitor to the domesticate maize (*Z. mays* ssp. *mays*) (Matsuoka et al. 2002), is an annual widely distributed at lower elevations from Jalisco to Oaxaca (Sanchez G. et al. 1998; Ruiz, Sánchez G., and Aguilar S. 2001). We used polymerase chain reaction (PCR) to amplify genes from the same species-wide collections of *Z. diploperennis* (7–13 alleles from 8 accessions) and *parviglumis* (12–17 alleles from 13 accessions). These accessions were also used in previous studies of defense genes in these species (Tiffin 2004; Tiffin, Hacker, and Gaut 2004), and information on the sources of the accessions sampled is provided in these references. Because *diploperennis* and *parviglumis* are highly outcrossing, PCR products were cloned into pGemTA vectors (Promega, Madison, Wis.) prior to sequencing. We also attempted to amplify each of these genes from *Tripsacum dactyloides* (Poaceae) for use as an outgroup in analyses.

Singleton variants obtained from clones may represent true polymorphisms or may result from misincorporation by *Taq* polymerase. For this reason, all singletons were checked by sequencing multiple clones from at least two separate PCRs or from direct sequencing of PCR products. When singleton variants were not recovered from separate PCRs or from PCR templates, they were assumed to have arisen from misincorporation by *Taq* polymerase and were excluded from analyses. Prior to analyses, sequences were aligned by eye using BioEdit version 6.0.5 (Hall 1999). We combined these newly collected data with previously published data for five defense and four nondefense genes from *Z. diploperennis* (table 1; Tiffin and Gaut 2001*b*; Tiffin, Hacker, and Gaut 2004) and 1 defense and 10 nondefense genes from *parviglumis* (table 2; Eyre-Walker et al. 1998; Hilton and Gaut 1998; Zhang et al. 2002; Tenaillon et al. 2004). GenBank sequences used for primer design, amplification conditions, and primer sequences as well as GenBank accession numbers for sequences that are new to this study are provided in Table 1 of Supplementary Material online.

Statistical Analyses

Nucleotide diversity at each locus was estimated using both the average number of pairwise differences per site between sequences, π (Nei 1987), and the average number of segregating sites per site, θ (Watterson 1975), for both nonsynonymous and silent sites, separately. We also calculated haplotype diversity, H_d (as in Nei 1987, but replacing $2n$ with n), for each locus. The tests of Tajima (*D*, 1989) and

Table 1
Number of Sequences (N), Number of Haplotypes (H), Number of Segregating Sites (S), Gene Length, Haplotype Diversity (H_d), Replacement and Silent Site Diversity (π_{rep}, π_{silent}) of Defense and Nondefense Genes in *Zea diploperennis*

	N	H	S	Length	H_d	π_{rep}	π_{silent}	π_{rep}/π_{silent}
Defense								
chiA	8[a]	1	0	1,100	0	0	0	—
chiB	7[a]	6	32	1,100	0.952	0.007	0.017	0.412
chiI	9[a]	6	19	1,080	0.889	0.003	0.009	0.333
hag	8	6	13	651	0.929	0.004	0.012	0.311
mir1	9	5	20	971	0.806	0.007	0.009	0.819
mpi	13[b]	9	13	630	0.870	0.001	0.008	0.125
plt1	9	5	17	482	0.806	0.009	0.018	0.498
plt2	11	3	6	609	0.473	0.006	0.001	4.125
pr1	9	4	9	517	0.583	0.003	0.011	0.229
pr5	10	7	11	478	0.911	0.009	0.010	0.906
pr6	8	5	18	1,172	0.786	0.002	0.012	0.125
prms	8	2	11	699	0.250	0.001	0.007	0.186
rip1	8	7	35	1,135	0.964	0.008	0.017	0.439
rip2	8	5	11	630	0.893	0.006	0.010	0.557
wip1	10[b]	8	27	660	0.933	0.006	0.027	0.222
zlp	8	7	5	721	0.964	0.001	0.006	0.152
Nondefense								
adh1	9[c]	9	38	1,400	0.972	0.001	0.017	0.059
c1	14[c]	11	16	750	0.967	0.004	0.010	0.450
glb1	9[c]	6	21	1,200	0.889	0.006	0.012	0.500
waxy	10[c]	9	16	1,400	0.978	0.003	0.006	0.500

[a] Data from Tiffin (2004).
[b] Data from Tiffin, Hacker, and Gaut (2004).
[c] Data from Tiffin and Gaut (2001b).

Table 2
Number of Sequences (N), Number of Haplotypes (H), Number of Segregating Sites (S), Gene Length, Haplotype Diversity (H_d), Replacement and Silent Site Diversity (π_{rep}, π_{silent}) of Defense and Nondefense Genes *Zea mays* ssp. *parviglumis*

	N	H	S	Length	H_d	π_{rep}	π_{silent}	π_{rep}/π_{silent}
Defense								
chiA	13[a]	13	51	1,100	0.987	0.006	0.026	0.231
chiB	14[a]	14	54	1,100	1	0.008	0.025	0.320
chiI	15[a]	14	27	1,080	0.990	0.001	0.016	0.062
cpi	12	9	74	1,274	0.939	0.010	0.018	0.556
hag	15	15	37	613	1	0.006	0.035	0.171
mir1	12	12	65	937	1	0.016	0.030	0.533
mpi	17	9	9	523	0.912	0.005	0.004	1.250
plt1	13	13	42	491	1	0.005	0.034	0.147
plt2	14	13	24	813	0.989	0.003	0.008	0.375
pr1	16	12	15	658	0.967	0.005	0.016	0.297
pr5	13	11	19	479	0.974	0.008	0.021	0.381
pr6	14	14	26	1,110	1	0.005	0.011	0.455
prms	16	9	21	699	0.817	0.002	0.012	0.167
rip1	14	14	55	1,147	1	0.008	0.020	0.400
rip2	13	13	21	615	1	0.007	0.016	0.438
wip1	14[b]	9	25	458	0.990	0.006	0.026	0.280
zlp	14	12	14	727	0.923	0.002	0.012	0.167
Nondefense								
adh1	8[c]	7	59	1,261	0.964	0.001	0.028	0.036
bz2	12[d]	8	15	590	0.939	0.002	0.015	0.147
c1	11[e]	7	29	635	0.909	0.002	0.026	0.065
csu381	10[d]	10	29	917	1	n/a	0.014	—
csu1132	8[d]	7	42	531	0.964	n/a	0.020	—
d8	13[d]	13	46	791	1	0.002	0.037	0.065
glb1	8[f]	8	75	991	1	0.014	0.037	0.391
tb1	7[d]	7	88	2,702	1	0.002	0.016	0.160
ts2	10[d]	10	17	975	1	0.001	0.016	0.087
waxy	9[e]	9	36	1,221	1	0.002	0.016	0.101

[a] Data from Tiffin (2004).
[b] Data from Tiffin and Gaut (2001a).
[c] Data from Eyre-Walker et al. (1998).
[d] Data from Tenaillon et al. (2004).
[e] Data from Zhang et al. (2002).
[f] Data from Hilton and Gaut (1998).

Fu (*Fs*, 1997) were used to test whether the frequency distribution of polymorphic sites deviated from the neutral equilibrium expectation. The test of Fay and Wu (*H*, 2000) was used to test for an excess of high-frequency–derived variants, and the test of McDonald and Kreitman (MK, 1991) was used to test whether the ratio of replacement to synonymous substitutions is equal to the ratio of replacement to synonymous polymorphism; both tests incorporate information on divergence from the outgroup, *T. dactyloides*. Statistical significance for Fu's *Fs* and Fay and Wu's *H* was assessed using coalescent simulations of 10,000 genealogies based on the number of segregating sites and assuming no recombination. Analyses of diversity and tests of neutrality for individual genes were conducted in DnaSP 4.0 (J. Rozas and R. Rozas 1999). We used multilocus HKA tests (Hudson, Kreitman, and Aguadé 1997) as implemented in software available from Jody Hey (Rutgers University, http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm) to test for deviations from the null hypothesis of a correlation between intraspecific diversity and interspecific divergence for defense and nondefense genes separately. We also conducted MK tests on pooled data from all defense genes and pooled data from all nondefense genes (see Schlenke and Begun 2003).

To compare directly the evolutionary histories of defense to nondefense genes, we used three approaches. First, we compared levels of diversity based on H_d, π, and θ between defense and nondefense genes using nonparametric Wilcoxon two-sample tests. Second, we used contingency tests to directly compare the distribution of polymorphism and fixed differences at replacement and synonymous sites between defense and nondefense genes. Third, we used the newly developed maximum-likelihood HKA test of Wright and Charlesworth (2004) to test for evidence of selection acting on defense genes by comparing ratios of intraspecific diversity to interspecific divergence at defense and nondefense genes (implemented in the program MLHKA available from Stephen I. Wright, York University, http://www.yorku.ca/stephenw/). Tests were conducted by comparing the fit of a neutral model to a model allowing for selection on all defense genes (full-selection model). If the full-selection model provided a significantly higher likelihood compared to the neutral model, we conducted a second test to compare the full-selection model to a simpler model that allowed for selection only on defense genes that exhibited strong deviations from neutrality (i.e., $k > 2$ or $k < 0.5$; see below). The neutral model is fit to the data by fitting a locus-specific θ, a species divergence time, T, and a selection parameter, k, which is fixed across all loci ($k_1 = k_2 = \cdots k_r = 1$). A model allowing for selection on defense genes also fits a separate θ for each locus but differs from the neutral model in allowing separate k values for each defense locus. The k values for reference (nondefense) genes in the selection models are fixed ($k = 1$) as in the neutral model (Wright and
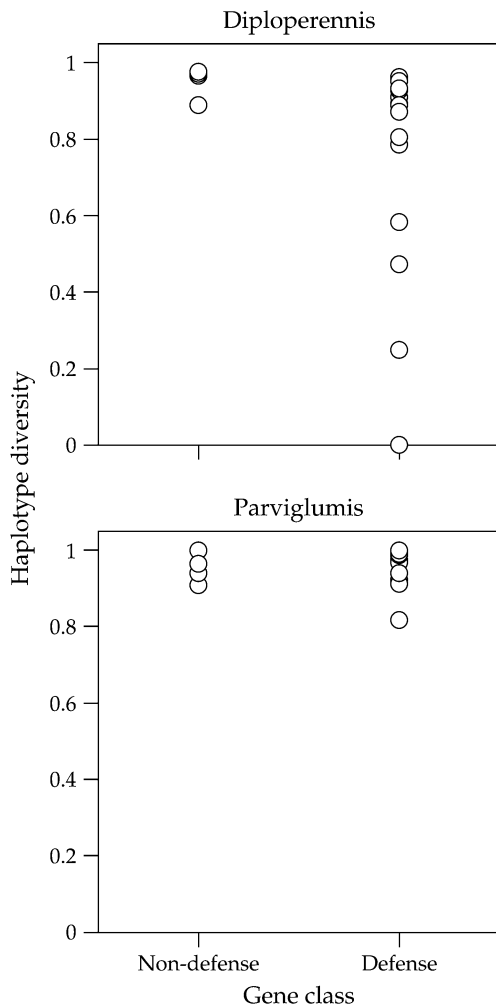
Fig. 1.—Haplotype diversity in defense and non-defense genes in *Zea diploperennis* and *Zea mays* ssp. *parviglumis.*

Fig. 2.—The relationship between π_{rep} and π_{syn} for defense and non-defense genes in *Zea diploperennis* and *Zea mays* ssp. *parviglumis*. The solid line indicates $\pi_{rep}/\pi_{syn} = 1$.

Charlesworth 2004). Regardless of the selection parameters, all models assume no recombination within loci, free recombination between loci, the standard neutral model of coalescence in a panmictic population, and the likelihood of the model is maximized using a Markov chain Monte Carlo approach (Wright and Charlesworth 2004). Preliminary analyses revealed that chains run for 1 or 10 million steps converged on very similar likelihoods, and therefore, we report on models that were fit with 1 million steps. Each model was fit using five independent runs with a different random-number seed. We used the mean likelihood and mean value of *k* averaged across the five independent runs of each model to evaluate whether selection had acted on defense genes. The goodness of fit of the different models was compared using likelihood-ratio tests where the difference in twice the log-likelihood between models was compared to a χ^2 distribution.

Results
Sequence Diversity

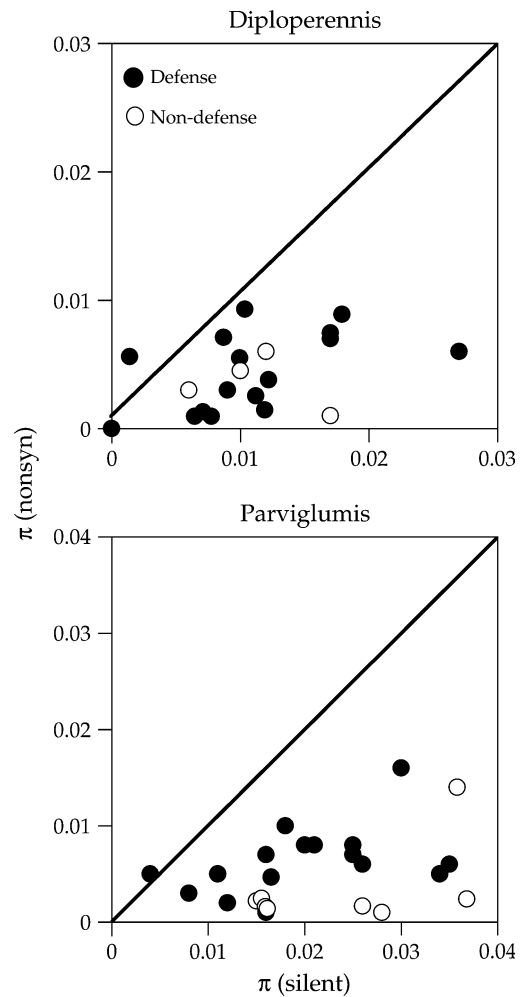We compared genetic diversity between defense and nondefense genes within each species by examining haplo-type diversity, H_d, the average pairwise nucleotide differences between sequences, π, and the average number of segregating sites per site, θ. In *diploperennis*, haplotype diversity (H_d) was significantly lower for defense than nondefense genes (fig. 1; mean $\pm$ SE for H_d: 0.75 $\pm$ 0.07 vs. 0.95 $\pm$ 0.02; Wilcoxon two-sample test, $P = 0.02$). Although differences in H_d between gene classes may arise because of differences in the number of segregating sites, we did not find significantly fewer segregating sites in defense (mean S = 15.44 $\pm$ 2.40) compared to nondefense genes (mean S = 22.75 $\pm$ 5.22, Wilcoxon two-sample test, $P = 0.17$). Nevertheless, this difference in H_d should be interpreted with caution because the sample size of nondefense genes was small and the mean number of segregating sites was higher in nondefense genes (table 1). The average pairwise number of nucleotide differences per site, π, measured using either nonsynonymous (π_{rep}) or silent sites (π_{silent}) in *diploperennis* did not differ significantly between defense and nondefense genes (mean π_{rep}: 0.0044 vs. 0.0036, Wilcoxon two-sample test, $P = 0.705$; mean π_{silent}: 0.0103 vs. 0.0112, $P = 0.720$). We also did not find differences between gene classes in π_{rep}/π_{silent} (fig. 2; Wilcoxon
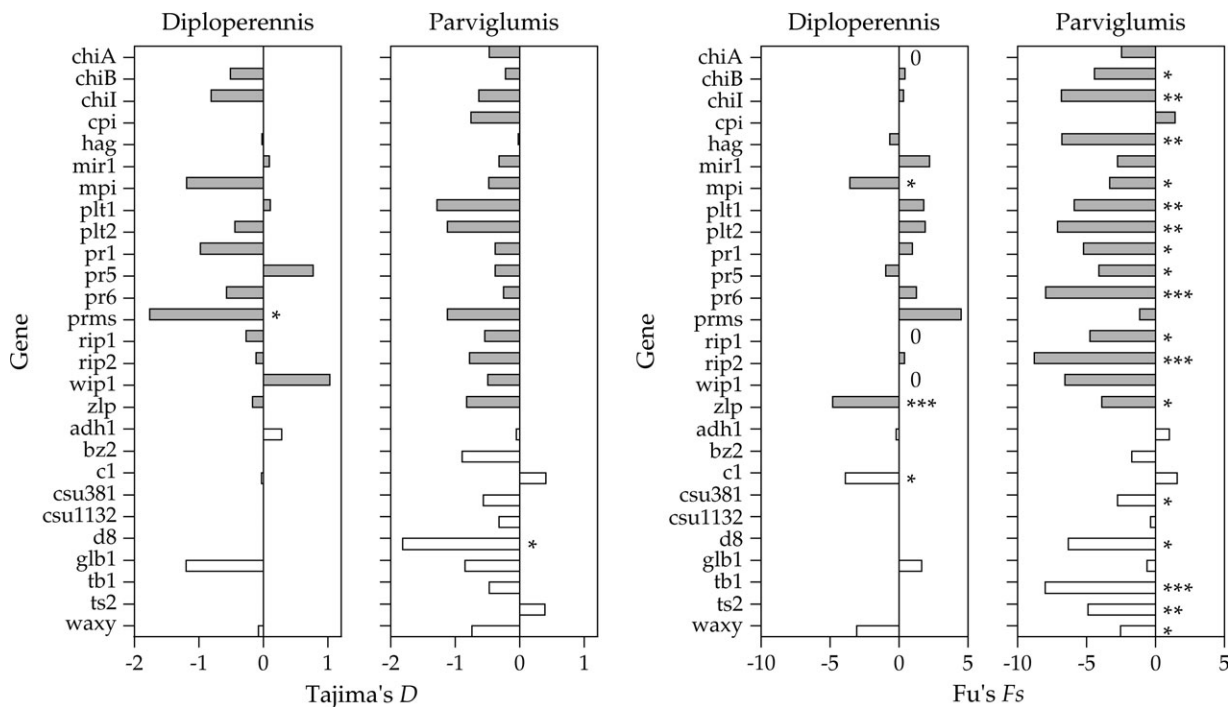
Fɪɢ. 3.—Tajima's *D* and Fu's *Fs* values calculated for individual defense (shaded bars) and nondefense genes (open bars) in *Zea diploperennis* and *Zea mays* ssp. *parviglumis*. Levels of statistical significance are indicated by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Values of Tajima's *D* and Fu's *Fs* are provided in Table 2 of the Supplementary Material online.

two-sample test, $P = 0.841$) or in nucleotide diversity measured using θ for either nonsynonymous or silent sites ($P = 0.925$ for both tests).

In *parviglumis*, we found no significant difference in haplotype diversity at defense compared to nondefense genes (fig. 1; $H_d = 0.97$ vs. 0.98; Wilcoxon two-sample test, $P = 0.65$). However, nucleotide diversity for replacement sites, π_{rep}, was significantly greater for defense compared to nondefense genes (fig. 2; 0.0059 vs. 0.0033; Wilcoxon two-sample test, $P = 0.018$), but π_{silent} did not differ significantly between gene classes (π_{silent}: 0.0190 vs. 0.0224; Wilcoxon two-sample test, $P = 0.325$). The difference in diversity at replacement sites is reflected in a significantly higher π_{rep}/π_{silent} at defense than nondefense genes (fig. 2; 0.366 vs. 0.132; Wilcoxon two-sample test, $P = 0.004$). A comparison of θ between gene classes also showed evidence of elevated diversity at non-synonymous sites ($P = 0.009$) but not silent sites ($P = 0.563$) in defense genes.

Tests of Neutral Evolution Conducted on Individual Genes

We applied four commonly used tests of neutrality, Tajima's *D*, Fu's *Fs*, Fay and Wu's *H*, and the MK test, to each of the defense genes individually. For *diploperennis*, Tajima's *D* was significantly negative for one gene, *prms* ($D = -1.76$, $P < 0.05$), and Fu's *Fs* was significantly negative for two defense genes, *mpi* and *zlp* (*mpi*: $Fs = -3.57$, $P < 0.05$; *zlp*: $Fs = -4.80$, $P < 0.001$; fig. 3; see also Table 2 of Supplementary Material online). For *chiA*, an absence of segregating sites indicates that this gene may have also experienced a recent selective sweep

although the lack of segregating sites prevents calculation of either Tajima's *D* or Fu's *Fs*. Across gene classes, however, we did not detect a significant difference in Tajima's *D* between defense and nondefense genes (Wilcoxon two-sample test, $P = 0.689$); estimates of Fu's *Fs* could not be compared between gene classes because values of *Fs* scale with the number of segregating sites. Fay and Wu's *H* was not significantly different from expectation under neutrality for any of the 11 *diploperennis* genes for which we had an outgroup sequence (see Table 2 of Supplementary Material online). Similarly, MK tests provided evidence of nonneutrality in only one gene, *chiB*, a result previously reported in Tiffin (2004). Multilocus HKA tests corroborated evidence for the apparent heterogeneity in evolutionary histories of defense genes revealed by single-locus tests ($P = 0.042$). Based on marginal χ^2 deviations, three of seven defense loci appear to be responsible for this heterogeneity: two loci (*chiA*, *mpi*) showed reduced levels of polymorphism and elevated divergence, while one locus (*rip1*) showed elevated levels of polymorphism and reduced divergence. We found no evidence for significant heterogeneity among nondefense genes ($P = 0.822$); however, it should be noted that the small number of nondefense genes included in the analysis means that there was little power to detect heterogeneous patterns of diversity.

For *parviglumis*, Tajima's *D* did not deviate from neutral expectation for any defense gene. By contrast, Fu's *Fs*, a test that is particularly sensitive to the effects of population expansion (Fu 1997), was significantly negative for 12 of 17 defense and 5 of 10 nondefense genes in *parviglumis*. Consistent with results from individual genes, the set of defense genes did not differ significantly from the

Table 3
Results of MK Tests for Data Pooled Within Gene Classes and Comparisons of the Distribution of Polymorphism and Divergence for Replacement and Synonymous Sites Between Gene Classes[a]

| | Polymorphism | | Divergence | | MK | Defense Versus Nondefense | | |
| | | | | | | Polymorphism | Divergence | All |
	Repl[b]	Syn[c]	Repl	Syn	χ^2 (P)	χ^2 (P)	χ^2 (P)	χ^2 (P)
diploperennis								
Defense	28	37	56	56	0.791 (0.374)	0.362 (0.547)	1.271 (0.260)	1.799 (0.615)
Nondefense	22	23	29	41	0.618 (0.432)			
parviglumis								
Defense	76	66	59	59	0.320 (0.572)	16.984 (<0.001)	2.123 (0.145)	19.295 (<0.001)
Nondefense	45	106	47	69	3.336 (0.068)			

[a] Only those genes for which we were able to obtain a sequence from the outgroup, *Tripsacum dactyloides*, were included in the analyses. Defense genes: *chiA*, *chiB*, *cpi*, *hag*, *mpi*, *pr1*, *rip1*, and *wip1*. Nondefense genes: *adh1*, *bz2*, *c1*, *csu381*, *csu1132*, *d8*, *glb1*, *tb1*, *ts2*, and *waxy*.
[b] Number of replacement polymorphisms.
[c] Number of synonymous polymorphisms.

set of nondefense genes for Tajima's *D* (Wilcoxon two-sample test, $P = 0.688$). The common rejection of the neutral model in both gene classes by Fu's *Fs* test, as well as consistently negative values of both Tajima's *D* (25 of 27 genes) and Fu's *Fs* (24 of 27 genes), suggests that demographic history has had a strong influence on patterns of diversity in *parviglumis* (fig. 3). Fay and Wu's *H* test, which could be conducted only for those genes for which we had an outgroup sequence (18 genes), was significantly negative for one defense gene in *parviglumis*, *cpi2* (Table 2 of Supplementary Material online). MK tests were not significant for any individual gene. Similarly, multilocus HKA tests revealed no evidence for significant heterogeneity in the evolutionary history of defense genes ($P = 0.931$) or nondefense genes ($P = 0.388$).

Comparative Multilocus Tests of Neutrality Using Defense and Nondefense Genes

MK tests conducted on data pooled for each gene class revealed no evidence for selection having acted on either defense or nondefense genes in either species (table 3). However, a 4×2 contingency test comparing defense to nondefense genes revealed significant heterogeneity in the distribution of fixed and polymorphic replacement and synonymous sites in *parviglumis* (table 3; $P < 0.001$) but not *diploperennis* ($P = 0.615$). Partitioning the contingency table for *parviglumis* between polymor-

phism and divergence revealed that significant heterogeneity in the 4×2 test was due mainly to heterogeneity in the ratio of replacement to synonymous polymorphic sites between gene classes ($P < 0.001$) and not the ratio of replacement to synonymous fixed differences between gene classes ($P = 0.145$). This significant heterogeneity in polymorphism could be due to any of the cells in the contingency table. Nevertheless, assuming that nondefense genes represent expectations under neutrality, then significance is most likely due to an excess of replacement polymorphism in defense genes (table 3), a finding consistent with the significantly elevated levels of π_{rep} and π_{rep}/π_{silent} at defense compared to nondefense genes.

The standard multilocus HKA test (results reported earlier) indicated that not all defense genes have experienced similar selective histories within *diploperennis*; however, it does not provide a direct test of whether selection has shaped diversity at defense loci differently than at nondefense loci. To test this, we used the maximum-likelihood HKA test of Wright and Charlesworth (2004) to compare the fit of a neutral model (selection parameter, $k = 1$ for all loci) to a model in which selection was allowed to act on defense (variable k) but not nondefense genes. For *diploperennis*, a likelihood-ratio test revealed that the selection model fit the data significantly better than the neutral model (table 4; $\chi^2 = 21.52$, df = 7, $P = 0.003$) providing evidence for the action of selection on defense genes. To test whether the rejection of the neutral model was due to all or only

Table 4
Results from Maximum-Likelihood HKA Tests Comparing the Fit of a Neutral Model to Models Allowing for Selection on Either Defense Loci or Nondefense loci

Model	ln *L*	Model Comparison	Likelihood-Ratio Statistic (df)	P
diploperennis				
A Neutral	−64.32			
B Selection, 7 defense loci	−53.56	A versus B	21.52 (7)	0.003
C Selection, 4 defense loci	−53.94	A versus C	20.77 (4)	<0.001
		B versus C	0.76 (3)	0.859
D Selection, 4 nondefense loci	−63.94	A versus D	0.76 (4)	0.944
parviglumis				
A Neutral	−89.76			
B Selection, 8 defense loci	−89.94	A versus B	0.36 (8)	0.999
C Selection, 7 nondefense loci	−87.84	A versus C	3.83 (7)	0.799

a subset of the defense genes, we then compared the full-selection model to a model that allowed for selection only on the four loci identified by the first selection model as putative targets of selection (*chiA* and *mpi*, both with $k < 0.33$, and *pr1* and *rip1*, both with $k > 2.0$; *k* measures the relationship between polymorphism and divergence and is expected to be 1 under neutrality). This reduced model also provided a significant improvement over the neutral model ($\chi^2 = 20.77$, df = 4, $P < 0.001$). The full-selection model did not, however, provide a significantly better fit than the reduced-selection model ($\chi^2 = 0.76$, df = 3, $P = 0.859$). Therefore, it appears as though a subset of the defense loci (*chiA*, *mpi*, *pr1*, and *rip1*) is responsible for driving the significant difference in the evolutionary history of defense versus nondefense genes. Estimates of the selection parameter, *k*, indicated that polymorphism was elevated more than twofold relative to neutral (nondefense gene) expectations for *rip1* and *pr1* ($k = 2.12$ and 2.86, respectively); whereas, polymorphism was approximately one-fourth of neutral expectations in *mpi* ($k = 0.26$) and strongly reduced for *chiA* ($k = 0$). To confirm that significant heterogeneity is restricted to defense genes, we also tested whether a model allowing for selection on nondefense genes (while constraining the defense genes to evolve neutrally) provides an improved fit over a strictly neutral model. This model did not fit the data significantly better than a neutral model regardless of whether all defense genes or only those not identified as having significant values of *k* were used as reference loci (both $P > 0.90$). Moreover, the *k* values for the four defense genes identified as having been shaped by selection fell outside the range of *k* values for the nondefense genes (range = 0.85–1.89). In contrast to *diploperennis*, a model allowing for selection on all defense genes did not provide a significantly better fit to the data than a neutral model for *parviglumis* (table 4; $\chi^2 = 0.36$, df = 8, $P = 0.99$). Similarly a model allowing for selection on nondefense genes, but not defense genes, did not fit the data significantly better than a neutral model ($\chi^2 = 3.83$, df = 7, $P = 0.80$).

Discussion

In order to further our understanding of the evolutionary history of plant defense genes, we examined the present-day diversity of 16 defense genes in *Z. diploperennis* and 17 defense genes in *parviglumis*. The defense genes we sampled encode proteins with diverse biochemical functions, but all are classified as pathogenesis-related proteins and are potentially important in inhibiting attack by pathogens or herbivores. Our specific objectives were twofold: to understand the nature and extent of variation among defense genes in patterns of past selection and to test whether defense genes, as a class, have experienced a different selective history than nondefense genes.

For *Z. diploperennis*, we found significant heterogeneity in the evolutionary histories of defense genes. Specifically, multilocus tests of homogeneity in the ratio of polymorphism to divergence identified two genes (*chiA* and *mpi*) with low diversity relative to divergence and two genes (*pr1* and *rip1*) that harbor an excess of intraspecific polymorphism relative to divergence.

The reduced diversity found at the chitinase gene, *chiA*, and protease inhibitor, *mpi*, are consistent with a history of positive selection on these or closely linked loci. The possibility of positive selection is also supported by the lack of segregating sites at *chiA* (Tiffin 2004) and the significantly negative value of Fu's *Fs* for *mpi*. There was also evidence suggestive of positive selection from two other genes for which HKA tests could not be performed. A significantly negative Fu's *Fs* was detected for the putative dual α-amylase trypsin inhibitor, *zlp*, and a significantly negative Tajima's *D* was detected for the *pr1*-like *prms*. It is important to note that two of 15 defense and one of 4 nondefense genes had significantly negative values for Fu's *Fs*, suggesting that demographic forces may be responsible for the rejection of the neutral equilibrium model. However, if demographic forces are responsible, they do not appear to have affected diversity across the genome— 12 of 19 values of *Fs* were positive. Similarly, the single significantly negative Tajima's *D* value may not reflect selection but simply a false positive, which is possible given that we conducted 19 separate tests in *Z. diploperennis*. The evidence of positive selection acting on *chiA* and *mpi* is consistent with expectations from coevolutionary arms-race models (Dawkins and Krebs 1979), which predict recurrent selective sweeps on defense genes. It is also possible, however, that these genes are not on a path to fixation as classic arms-race models predict. Rather, they may be evolving in a manner consistent with expectation from allelic cycling models (May and Anderson 1983; Seger 1988, 1990; Frank 1993; Stahl et al. 1999) which predict that periods of positive selection will drive the selected alleles to high frequency but that the selective environment will change before the alleles reach fixation.

In contrast to the defense genes that show reduced diversity as expected under positive selection, *pr1* and *rip1* harbor an excess of diversity relative to divergence, a pattern often interpreted as evidence of balancing selection. However, we found no other evidence for balancing selection acting on these loci—Tajima's *D* values are negative, linkage disequilibrium is not more extensive at these than at other loci (data not shown), and genealogies do not show evidence for distinct allelic classes (not shown). Therefore, it does not appear that selection has maintained a long-lived polymorphism at these loci. It is, however, possible that the elevated levels of diversity reflect a more complicated form of enemy-imposed selection or antagonistic coevolution, perhaps associated with ongoing or periodic selection from multiple genetically distinct enemies. Regardless of whether the evidence for nonneutral evolution provides support for any particular coevolutionary model, our results do provide evidence for substantial variability in the evolutionary histories of downstream components of plant defense response (i.e., PR genes).

Although the significant heterogeneity we detected in intraspecific diversity may reflect the action of selection, these differences may also be due to indirect effects of recombination on genetic diversity (Begun and Aquadro 1993). For example, *chiA* and *mpi* may be located in regions of low recombination and thus be more affected

by background selection (B. Charlesworth, Morgan, and D. Charlesworth 1993; Hudson and Kaplan 1995) and hitchhiking (Maynard-Smith and Haigh 1974; Kaplan, Hudson, and Langley 1989), whereas *pr1* and *rip1* may be located in regions of high recombination. Under this scenario, the remainder of the defense and nondefense genes would be expected to reside in regions of "normal" recombination. Excluding recombination as an explanation for our results would require information on the recombinational landscape and the genomic location of each gene in *Z. diploperennis*, which is not available. However, two lines of evidence suggest that differences in rates of recombination do not explain the variation in diversity we detected. First, the estimated recombination rate, *R* (Hudson 1987), for *mpi* is higher than the average calculated for the defense genes, the estimate for *pr1* is lower than the average, and the estimate for *rip1* is toward the upper end of the range but not markedly different from other genes (Supplementary Material Table 2, *chiA* harbors no polymorphic sites and thus *R* could not be calculated). Second, if recombination strongly affects diversity, then we might expect to find low diversity at *chiA* and *mpi* and high diversity at *pr1* and *rip1* in the closely related species *parviglumis*, which we do not (Supplementary Material Table 2). Finally, we note that there is some evidence that the positive relationship between recombination rate and nucleotide diversity may not be as strong in *Zea* as has been found in other systems (Tenaillon et al. 2002).

In contrast to *Z. diploperennis*, in which a subset of defense genes may have experienced recent selection, we found no evidence for selection having acted on individual PR genes in *parviglumis*. Although our ability to detect selection may have been limited by small sample sizes, we did find that patterns of intraspecific diversity were considerably different in the two species. In particular, we found a significant excess of replacement polymorphism segregating at defense genes in *parviglumis* but not *diploperennis*. Specifically, π_{rep} and π_{rep}/π_{silent} were higher at defense than nondefense genes, and contingency tests revealed significant heterogeneity in the distribution of fixed and polymorphic sites in defense compared to nondefense genes, apparently due to an excess of replacement polymorphism in defense genes. Interestingly, elevated replacement polymorphism has been detected at other genes involved in mediating antagonistic coevolution, including a pathogen recognition gene in *A. thaliana* (*rpp13*, Rose et al. 2004), a protease inhibitor in European Aspen (Ingvarsson, 2005), inducible antibacterial peptide genes in *D. melanogastor* (Lazzaro and Clark 2003), and genes involved in sperm competition in several *Drosophila* species (Begun et al. 2000; Kern, Jones, and Begun 2004). At least four processes may explain the elevated levels of replacement diversity we found in *parviglumis*: relaxed selective constraints, stable balanced polymorphisms, transient or episodic selection that favors specific alleles only when those alleles are rare, or geographically variable selection on defense genes.

Higher levels of replacement polymorphism in defense compared to nondefense genes could result from relaxed selective pressure on defense genes, allowing for an accumulation of slightly deleterious mutations. Selective constraints may be relaxed if there has been a change in

the functional importance of defense genes, either due to reductions in enemy population sizes, the evolution of counterdefense mechanisms in enemy populations, or the evolution of tolerance in the host plants. Although we cannot exclude the possibility of relaxed selection, two aspects of the data suggest that relaxed selective constraints are not the primary explanation for the elevated levels of replacement polymorphism. First, we found no null alleles or frameshift mutations in our sample of 239 defense alleles from *parviglumis*, making it unlikely that selection has been relaxed over any appreciable period of time. Second, the evidence for positive selection in *diploperennis* suggests that at least some defense genes are functional in this closely related species.

Higher levels of replacement polymorphism at defense genes may also reflect the action of selection maintaining polymorphism; however, we found little corroborating evidence for this hypothesis. Under simple models of balanced polymorphism, we expect to find elevated levels of polymorphism at silent sites surrounding the target of selection and high linkage disequilibrium (e.g., Kreitman and Hudson 1991; Richman, Uyenoyama, and Kohn 1996). Although both these patterns have been found at other defense loci at which polymorphism appears to be maintained by selection (e.g., Tian et al. 2002; Kroymann et al. 2003; Mauricio et al. 2003), neither was apparent in our data. In fact, the genetic association between polymorphic sites, estimated for each gene by *ZnS* (Kelly 1997), was greater, although not significantly so, in nondefense genes ($t = 1.82$, $P = 0.081$). In addition, Tajima's *D* is expected to be positive under simple balanced polymorphism models, but our analyses revealed negative Tajima's *D* for all 17 defense genes. These summary statistics, therefore, suggest that it is unlikely that balancing selection maintains amino acid diversity at these loci.

A third possible explanation for the excess of replacement polymorphisms is that defense genes experience transient episodes of positive selection. Specifically, rare allelic variants may rapidly increase in frequency when enemy pressure is strong and resistance enhances plant fitness but later may become selectively neutral due to enemy counteradaptation or a low frequency of attack, i.e., rare or novel allele advantage (Haldane 1949; Tiffin and Gaut 2001a; de Meaux and Mitchell-Olds 2003; Lazarro and Clark 2003). This pattern of selection differs from balancing selection in that polymorphism is not necessarily maintained by natural selection but rather accrues due to periodic bursts of positive selection.

Finally, elevated replacement polymorphism may result from population structure coupled with local selection on defense genes. Population structure as well as population expansion or contraction can cause the frequency of polymorphic sites to deviate from expectations under a neutral model (B. Charlesworth, Nordborg, and D. Charlesworth 1997; B. Charlesworth, D. Charlesworth, and Barton 2003) and result in significantly negative values of Tajima's *D* and Fu's *Fs* (Tajima 1989; Fu 1997; Wright and Gaut 2005). We found negative Tajima's *D* and Fu's *Fs* values for virtually all defense and nondefense genes sampled from *parviglumis*, suggesting that demographic factors have strongly influenced the frequency spectrum of

polymorphic sites (see also Zhang et al. 2002). Moreover, the present-day distribution of *parviglumis*, which is found in geographically distinct populations spread across south-central and southwestern Mexico (Ruiz, Sánchez G., and Aguilar S. 2001), as well as evidence for recent climate change in the region (Buckler, Pearsall, and Holtsford 1998) make it reasonable to think that patterns of polymorphism in *parviglumis* may be shaped by demographic forces and historical shifts in distribution and abundance. If population structure coupled with local selection is responsible for the significant excess of replacement polymorphisms we detected in *parviglumis*, then it may not be surprising that we found distinctly different patterns of diversity in *parviglumis* and *diploperennis*. Unlike *parviglumis*, with populations spread across south-central and southwestern Mexico, *diploperennis* has a limited geographic range (Sanchez G. et al. 1998; Ruiz, Sánchez G., and Aguilar S. 2001), and summary statistics based on the frequency spectrum of polymorphic sites reveal no evidence of population structure or nonequilibrium population dynamics (fig. 3).

Because the effect of demographic history should be independent of gene function, population expansion or population structure alone cannot account for the elevated replacement polymorphism we found at defense compared to nondefense loci. Geographically variable selection on defense genes, by contrast, may lead to the fixation of different amino acid–changing alleles in different partially isolated populations (Begun and Aquadro 1993; Slatkin and Wiehe 1998; Barton 2000) and therefore to high levels of replacement polymorphism in species-wide samples. Evidence for local adaptation in host-parasite interactions has been found in several systems (reviewed in Kaltz and Shykoff 1998; Van Zandt and Mopper 1998; Bergelson, Dwyer, and Emerson 2001; Kniskern and Rausher 2000), suggesting that spatial variation in selection and adaptive differentiation is at least plausible in *parviglumis*. Unfortunately, relatively little is known about the natural enemies that attack teosintes, let alone the patterns of selection imposed by these enemies or the geographic scale over which this selection acts in contemporary populations. Regardless of short-term ecological and evolutionary dynamics in contemporary populations, hierarchical spatial sampling of DNA sequence diversity should reveal whether local adaptation has been important in shaping defense gene diversity over longer periods of time.

Supplementary Material

Tables 1–3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

Literature Cited

Barton, N. H. 2000. Genetic hitchhiking. Philos. Trans. R. Soc. Lond. B **355**:1553–1562.

Begun, D. J., and C. F. Aquadro. 1993. African and North American populations of *Drosophila melanogaster* are very different at the DNA level. Nature **365**:548–550.

Begun, D. J., P. Whitley, B. L. Todd, H. M. Waldrip-Dail, and A. G. Clark. 2000. Molecular population genetics of male accessory gland proteins in *Drosophila*. Genetics **156**:1879–1888.

Bergelson, J., G. Dwyer, and J. J. Emerson. 2001. Models and data on plant-enemy coevolution. Annu. Rev. Genet. **35**:469–499.

Bergelson, J., M. Kreitman, E. A. Stahl, and D. Tian. 2001. Evolutionary dynamics of plant R-genes. Science **292**:2281–2285.

Bishop, J. G., A. M. Dean, and T. Mitchell-Olds. 2000. Rapid evolution in plant chitinases: molecular targets of selection in plant-pathogen coevolution. Proc. Natl. Acad. Sci. USA **97**:5322–5327.

Bishop, J. G., D. R. Ripoll, S. Bashir, C. M. B. Damasceno, J. D. Seeds, and J. K. C. Rose. 2005. Selection on *Glycine* β-1-3-endoglucanase genes differentially inhibited by a phytophthora glucanase inhibitor protein. Genetics **169**:1009–1019.

Buchel, A. S., and H. J. M. Linthorst. 1999. PR-1: a group of plant proteins induced upon pathogen infection. Pp. 21–47 *in* S. K. Datta and S. Muthukrishnan, eds. Pathogenesis-related proteins in plants. CRC Press, Boca Raton, Fla.

Buckler, E. S., D. M. Pearsall, and T. P. Holtsford. 1998. Climate, plant ecology, and central Mexican archaic subsistence. Curr. Anthropol. **39**:152–164.

Caicedo, A. L., B. A. Schaal, and B. A. Kunkel. 1999. Diversity and molecular evolution of the *Rps2* resistance gene in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **96**:302–306.

Charlesworth, B., D. Charlesworth, and N. H. Barton. 2003. The effects of genetic and geographic structure on neutral variation. Annu. Rev. Ecol. Syst. **34**:99–125.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1993. The effect of deleterious mutations on neutral molecular variation. Genetics **134**:1289–1303.

Charlesworth, B., M. Nordborg, and D. Charlesworth. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. Genet. Res. **70**:155–174.

Clauss, M. J., and T. Mitchell-Olds. 2003. Population genetics of tandem trypsin inhibitor genes in *Arabidopsis* species with contrasting ecology and life history. Mol. Ecol. **12**:1287–1299.

Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. Proc. R. Soc. Lond. B **205**:489–511.

de Meaux, J., and T. Mitchell-Olds. 2003. Evolution of plant resistance at the molecular level: ecological context of species interactions. Heredity **91**:345–352.

Eyre-Walker, A., R. L. Gaut, H. Hilton, D. L. Feldman, and B. S. Gaut. 1998. Investigation of the bottleneck leading to the domestication of maize. Proc. Natl. Acad. Sci. USA **95**:4441–4446.

Fay, J. C., and C.-I. Wu. 2000. Hitchhiking under positive Darwinian selection. Genetics **155**:1405–1413.

Frank, S. A. 1993. Coevolutionary genetics of plants and pathogens. Evol. Ecol. **7**:45–75.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics **147**:915–925.

Haldane, J. B. S. 1949. Disease and evolution. Ric. Sci. Suppl. A **19**:68–76.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and program for windows 95/98/NT. Nucleic Acids Symp. Ser. **41**:95–98.

Hilton, H., and B. S. Gaut. 1998. Speciation and domestication in maize and its wild relatives: evidence from the globulin-1 gene. Genetics **150**:863–872.

Hudson, R. R. 1987. Estimating the recombination parameter of a finite population model without selection. Genet. Res. **50**: 245–250.

Hudson, R. R., and N. L. Kaplan. 1995. Deleterious background selection with recombination. Genetics **141**:1605–1617.

Hudson, R. R., M. Kreitman, and M. Aguadé. 1997. A test of neutral molecular evolution based on nucleotide data. Genetics **116**:153–159.

Huynh, Q. K., C. M. Hironaka, E. B. Levine, C. E. Smith, J. R. Borgmeyer, and D. M. Shah. 1992. Antifungal proteins from plants: purification, molecular cloning, and antifungal properties of chitinases from maize seed. J. Biol. Chem. **267**:6635–6640.

Iltis, H. H., J. F. Doebley, R. Guzman, and B. Pazy. 1979. *Zea diploperennis* (Gramineae): a new teosinte from Mexico. Science **203**:186–188.

Ingvarsson, P. K. 2005. Molecular population genetics of herbivore-induced protease inhibitor genes in European Aspen (*Populus tremula* L., Salicaceae). Mol. Biol. Evol. **22**: 1802–1812.

Jensen, A. B., R. Leah, B. Chaudhry, and J. Mundy. 1999. Ribosome inactivating proteins: structure, function, and engineering. Pp. 235–245 *in* S. K. Datta and S. Muthukrishnan, eds. Pathogenesis-related proteins in plants. CRC Press, Boca Raton, Fla.

Jiggins, F. M., and G. D. D. Hurst. 2003. The evolution of parasite recognition genes in the innate immune system: purifying selection on *Drosophila melanogaster* peptidoglycan recognition proteins. J. Mol. Evol. **57**:598–605.

Kader, J. C. 1996. Lipid-transfer proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47**:627–654.

Kaltz, O., and J. A. Shykoff. 1998. Local adaptation in host-parasite systems. Heredity **81**:361–370.

Kaplan, N. L., R. R. Hudson, and C. H. Langley. 1989. The "hitch-hiking effect" revisited. Genetics **123**:887–899.

Kawabe, A., and N. T. Miyashita. 1999. DNA variation in the basic chitinase locus (*ChiB*) region of the wild plant *Arabidopsis thaliana*. Genetics **153**:1445–1453.

Kelly, J. K. 1997. A test of neutrality based on interlocus associations. Genetics **146**:1197–1206.

Kern, A. D., C. D. Jones, and D. J. Begun. 2004. Molecular population genetics of male accessory gland proteins in the *Drosophila simulans* complex. Genetics **167**:725–735.

Kniskern, J., and M. D. Rausher. 2000. Two modes of host-enemy coevolution. Popul. Ecol. **43**:3–14.

Kreitman, M., and R. R. Hudson. 1991. Inferring the evolutionary histories of *Adh* and the *Adh-dup* loci in *Drosophila melanogaster* from patterns of polymorphism and divergence. Genetics **127**:565–582.

Kroymann, J., S. Donnerhacke, D. Schnabelrauch, and T. Mitchell-Olds. 2003. Evolutionary dynamics of an *Arabidopsis* insect resistance quantitative trait locus. Proc. Natl. Acad. Sci. USA **100**:14587–14592.

Lazzaro, B. P., and A. G. Clark. 2003. Molecular population genetics of inducible antibacterial peptide genes in *Drosophila melanogaster*. Mol. Biol. Evol. **20**:914–923.

Matsuoka, Y., Y. Vigouroux, M. M. Goodman, J. Sanchez G., E. S. Buckler, and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. USA **99**:6080–6084.

Mauricio, R., E. A. Stahl, T. Korves, D. Tian, M. Kreitman, and J. Bergelson. 2003. Natural selection for polymorphism in the disease resistance gene *Rps2* of *Arabidopsis*. Genetics **163**:735–746.

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

Maynard-Smith, J. and J. Haigh. 1974. The hitch-hiking effect of a favorable gene. Genet. Res. **23**:23–35.

McDonald, J. H., and M. Kreitman. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature **3551**:652–654.

Menezes, H., and C. Jared. 2002. Immunity in plants and animals: common ends through different means using similar tools. Comp. Biochem. Physiol. C **132**:1–7.

Meyers, B. C., K. A. Shen, P. Rohani, B. S. Gaut, and R. W. Michelmore. 1998. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell **11**:1833–1846.

Mondragon-Palomino, M., B. Meyers, R. Michelmore, and B. Gaut. 2002. Patterns of positive selection in the complete NBS-LRR gene family of *Arabidopsis thaliana*. Genome Res. **12**:1305–1315.

Morris, S. W., B. Vernooij, S. Titatarn, M. Starrett, S. Thomas, C. C. Wiltse, R. A. Frederiksen, A. Bhandhafalck, S. Hulbert, and S. Uknes. 1998. Induced resistance responses in maize. Mol. Plant-Microbe Interact. **11**:643–658.

Murillo, I., L. Cavallarin, and B. San Segundo. 1997. The maize pathogenesis-related PRms protein localizes to plasmodesmata in maize radicles. Plant Cell **9**:145–156.

Muthukrishnan, S., G. H. Liang, H. N. Trick, and B. S. Gill. 2001. Pathogenesis-related proteins and their genes in cereals. Plant Cell Tissue Organ Cult. **64**:93–114.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nielsen, K., G. A. Payne, and R. S. Boston. 2001. Maize ribosome-inactivating protein inhibits normal development of *Aspergillus nidulans* and *Aspergillus flavus*. Mol. Plant-Microbe Interact. **14**:164–172.

Nimchuk, Z., T. Eulgem, B. F. Holt III, and J. L. Dangl. 2003. Recognition and response in the plant immune system. Annu. Rev. Genet. **37**:579–609.

Pechan, T., A. Cohen, W. P. Williams, and D. S. Luthe. 2002. Insect feeding mobilizes a unique plant defense protease that disrupts the peritrophic matrix of caterpillars. Proc. Natl. Acad. Sci. USA **99**:13319–13323.

Przeworski, M. 2002. The signature of positive selection at randomly chosen loci. Genetics **160**:1179–1189.

Rausher, M. D. 2001. Co-evolution and plant resistance to natural enemies. Nature **411**:857–864.

Richman, A. D., M. K. Uyenoyama, and J. R. Kohn. 1996. Allelic diversity and gene genealogy at the self-incompatibility locus in the Solanaceae. Science **273**:1212–1216.

Rose, L. E., P. D. Bittner-Eddy, C. H. Langley, E. B. Holub, R. W. Michelmore, and J. L. Beynon. 2004. The maintenance of extreme amino acid diversity at the disease resistance gene, *RPP13*, in *Arabidopsis thaliana*. Genetics **166**:1517–1527.

Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics **15**:174–175.

Ruiz, J. A., J. Sánchez G., and M. Aguilar S. 2001. Potential geographical distribution of teosinte in Mexico: a GIS approach. Maydica **46**:105–110.

Ryan, C. A. 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. Annu. Rev. Phytopathol. **28**:425–449.

Sanchez G., J. G. Sanchez, Y. Kato, M. Aguilar S., H. M. Hernandez C., A. Lopez R., and J. A. Ruiz C. 1998. Distribución y caracterización del teocintle. Libro Técnico No. 2. INIFAP-CIRPAC, ed. Conexión Gráfica, Guadalajara, Jalisco, México, 149 p.

Schlenke, T. A., and D. J. Begun. 2003. Natural selection drives *Drosophila* immune system evolution. Genetics **164**:1471–1480.

Schlumbaum, A., F. Mauch, U. Vogelli, and T. Boller. 1986. Plant chitinases are potent inhibitors of plant growth. Nature **324**:365–367.

Seger, J. 1988. Dynamics of some simple host-parasite models with more than two genotypes in each species. Philos. Trans. R. Soc. Lond. B **319**:541–555.

———. 1990. Evolution of exploiter-victim relationships. Pp. 3–25 *in* M. J. Crawley, ed. Natural enemies: the population biology of predators, parasites, and diseases. Blackwell Scientific, London.

Slatkin, M., and T. Wiehe. 1998. Genetic hitch-hiking in a subdivided population. Genet. Res. **71**:155–160.

Stahl, E. A., G. Dwyer, R. Mauricio, M. Kreitman, and J. Bergelson. 1999. Dynamics of disease resistance polymorphism at the Rpm1 locus of *Arabidopsis*. Nature **400**:667–671.

Stotz, H. U., J. G. Bishop, C. W. Bergmann, M. Koch, P. Albersheim, A. G. Darvill, and J. M. Labavitch. 2000. Identification of target amino acids that affect interactions of fungal polygalacturonases and their plant inhibitors. Physiol. Mol. Plant Pathol. **56**:117–130.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585–595.

Tenaillon, M. I., M. C. Sawkins, L. K. Anderson, S. M. Stack, J. Doebley, and B. S. Gaut. 2002. Patterns of diversity and recombination along chromosome 1 of maize (*Zea mays* ssp *mays* L.). Genetics **162**:1401–1413.

Tenaillon, M. I., J. U'ren, O. Tenaillon, and B. S. Gaut. 2004. Selection versus demography: a multi-locus investigation of the domestication process in maize. Mol. Biol. Evol. **21**:1214–1225.

Tian, D. C., H. Araki, E. A. Stahl, J. Bergelson, and M. Kreitman. 2002. Signature of balancing selection in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **99**:11525–11530.

Tiffin, P. 2004. Comparative evolutionary histories of chitinase genes in the genus *Zea* and the family Poaceae. Genetics **167**:1331–1340.

Tiffin, P., and B. S. Gaut. 2001*a*. Molecular evolution of the wound-induced serine protease inhibitor *wip1* in *Zea* and related genera. Mol. Biol. Evol. **18**:2092–2101.

———. 2001*b*. Sequence diversity in the autotetraploid *Zea perennis* and the closely related diploid *Zea diploperennis*: insights from four nuclear loci. Genetics **158**:401–412.

Tiffin, P., R. Hacker, and B. S. Gaut. 2004. Population genetic evidence for rapid changes in intraspecific diversity and allelic cycling of a specialist defense gene in *Zea*. Genetics **168**:425–434.

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

Van Zandt, P. A., and S. Mopper. 1998. A meta-analysis of adaptive deme formation in phytophagous insect populations. Am. Nat. **152**:595–604.

Velazhahan, R., S. K. Datta, and S. Muthukrishnan. 1999. The PR-5 family: thaumatin-like proteins. Pp. 107–129 *in* S. K. Datta and S. Muthukrishnan, eds. Pathogenesis-related proteins in plants. CRC Press, Boca Raton, Fla.

Wang, G.-L., D.-L. Ruan, W.-Y. Song et al. (12 co-authors). 1998. *Xa21D* encodes a receptor-like molecule with a leucine rich repeat doman that determines race-specific recognition and is subject to adaptive evolution. Plant Cell **10**:765–779.

Ward, E. R., S. Uknes, S. C. Williams, S. Dincher, D. L. Wiederhold, D. C. Alexander, P. Ahl-Goy, J. P. Metraux, and J. A. Ryals. 1991. Coordinated gene activity in response to agents that induce systemic aquired resistance. Plant Cell **3**:1085–1094.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. **7**:188–193.

Wright, S. I., and B. Charlesworth. 2004. The HKA test revisited: a maximum-likelihood-ratio test of the standard neutral model. Genetics **168**:1071–1076.

Wright, S. I., and B. S. Gaut. 2005. Molecular population genetics and the search for adaptive evolution in plants. Mol. Biol. Evol. **22**:506–519.

Zhang, L., A. S. Peek, D. Dunams, and B. S. Gaut. 2002. Population genetics of duplicated disease-defense genes, *hm1* and *hm2*, in maize (*Zea mays* ssp. *mays* L.) and its wild ancestor (*Zea mays* ssp. *parviglumis*). Genetics **162**:851–860.