Natural selection mapping of the warfarin-resistance gene

Michael H. Kohn*†, Hans-Joachim Pelz‡, and Robert K. Wayne*

*Department of Organismic Biology, Ecology, and Evolution (OBEE), University of California, Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606; and ‡Federal Biological Research Center for Agriculture and Forestry, Institute for Nematology and Vertebrate Research, Toppheideweg 88, D-48161 Münster, Germany

In theory, genes under natural selection can be revealed by unique patterns of linkage disequilibrium (LD) and polymorphism at physically linked loci. However, given the effects of recombination and mutation, the physical extent and persistence of LD patterns in natural populations is uncertain. To assess the LD signature of selection, we survey variation in 26 microsatellite loci spanning an ≈32-cM region that includes the warfarin-resistance gene (*Rw*) in five wild rat populations having resistance levels between 0 and 95%. We find a high frequency of heterozygote deficiency at microsatellite loci in resistant populations, and a negative association between gene diversity (H) and resistance. Contrary to previous studies, these data suggest that directional rather than overdominant selection may predominate during periods of intense anticoagulant treatment. In highly resistant populations, extensive LD was observed over a chromosome segment spanning ≈14% of rat chromosome 1. In contrast, LD in a moderately resistant population was more localized and, in conjunction with likelihood ratios, allowed assignment of *Rw* to a 2.2-cM interval. Within this genomic window, a diagnostic marker, *D1Rat219*, assigned 91% of rats to the correct resistance category. These results further demonstrate that "natural selection mapping" in field populations can detect and map major fitness-related genes, and question overdominance as the predominant mode of selection in anticoagulant-resistant rat populations.

Selection can be intense in natural populations and cause substantial change over brief time intervals and small geographical scales that overwhelms the effects of drift, mutation, and migration (1, 2). Under a variety of models, polymorphism of neutral genetic markers can deviate from equilibrium expectations when they are in close physical linkage to genes under selection (3–10). Initially, after an episode of intense directional selection (a selective sweep), preexisting alleles at neutral markers physically linked to the favored gene are reduced in gene diversity (H), and linkage disequilibrium (LD) is increased (3, 6, 8). During this transient stage, population size and genetic polymorphism should be uncoupled (2, 5). Hence, genes under selection potentially may be revealed and genetically mapped by patterns of gene diversity and linkage disequilibrium at associated marker loci, such as microsatellites (11, 12). However, the persistence of such genomic signatures of selection in natural populations may be too ephemeral to be detected and used for genetic mapping considering that equilibrium conditions can be restored rapidly by recombination and mutation (9, 13).

The marker-based detection and mapping of major fitness-related genes usually requires that genealogical information be obtained from segregating experimental populations. To bypass this requirement, and to study the underlying genetic architecture of quantitative character variation, a few recent studies of natural populations have searched and detected distinct patterns of LD whose origin and maintenance was promoted by selection or population structure. For example, LD was found among closely spaced polymorphic nucleotide sites of cloned genes (14–18), within the major histocompatibility complex-spanning region (9, 12, 19), over limited chromosomal regions of low recombination (20), or across large genomic segments in naturally occurring hybrid populations (21).

Here, to test whether genomic screens for LD in field populations can reveal genes under selection, we studied genetically determined resistance to anticoagulant rodenticides in the Norway rat (*Rattus norvegicus*). This system is a well documented example of an evolutionary response to natural selection (22–24) and has several other characteristics that make it well suited for testing the approach. Resistance has a simple genetic basis (25, 26), with a physiological effect that can be measured with a blood clotting response (BCR) test (27, 28). *Rw* has been localized to an ≈6-cM interval on rat chromosome 1 (29, 30), and within this region there is an abundance of positionally mapped polymorphic microsatellite markers (31). Anticoagulant selection is intense, with on average 95% mortality (range ≈60–100%) (32). Population resistance levels can change rapidly with anticoagulant application (22–24, 33), and the frequency and intensity of anticoagulant selection varies among populations (22–24, 33).

Materials and Methods

Origin of Rats, Resistance Testing, and Population Size Index. We sampled rats from an area in north-western Germany where, beginning in 1990, rat infestations caused increasing control problems despite the use of anticoagulant rodenticides (Fig. 1; ref. 33). An extensive field survey using BCR tests on live trapped rats revealed an area of more than 8,000 km² that harbored populations that displayed resistance to anticoagulant compounds (33). Rats from four populations, WU, BK, PS, and HT, were live-trapped in and around farm buildings from this resistance area (Fig. 1). A control population, LH, in which no resistance has been reported to date, was sampled approximately 300 km from the resistance area (Fig. 1). Resistance to the anticoagulants warfarin, bromadiolone, and difenacoum was measured by the BCR method that utilizes the relative delay in coagulation time after administration of a diagnostic dose (33, 34). Rats were sexed, weighted, and subsequently caged individually at the animal husbandry facilities of the Institute for Nematology and Vertebrate Research of the Federal Biological Research Center for Agriculture and Forestry, Münster, Germany (BBA). BCR measurements of rats were compared with untreated controls and expressed in percent coagulation activity retained. For warfarin, bromadiolone, and difenacoum, individuals with a percent coagulation activity >17.5%, >12.5%, and >12.5% of the normal value, respectively, were classified as resistant (33, 34). Population resistance was expressed in percent resistant rats (Fig. 1). Tests followed the European and Mediterranean Plant Protection Organization (EPPO) guidelines

---

EVOLUTION
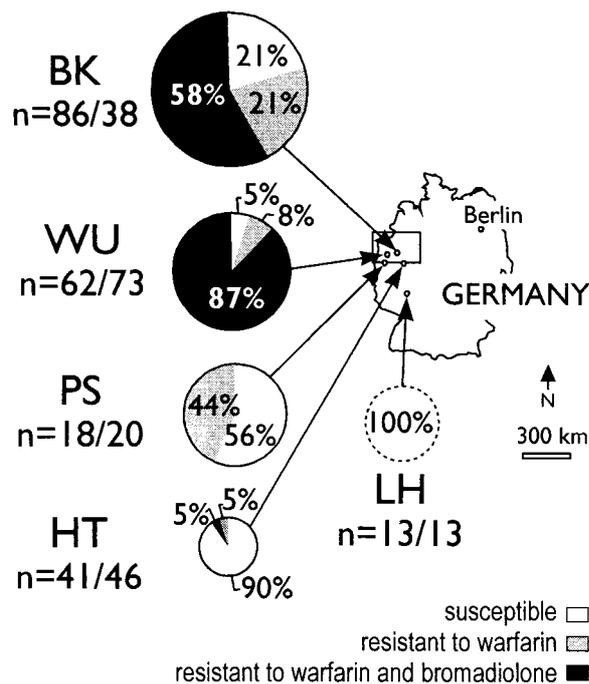
Fig. 1. Origin of rats, their resistance level, and relative population size. Collection sites and the nearest town (in parentheses) are as follows: BK (Olfen); WU (Stadtlohn); PS (Dorsten); HT (Drensteinfurt); all are located within the previously described resistance area that includes the city of Münster (box; ref. 33). Sampling site LH (Ludwigshafen) was located outside the resistance area. The frequencies of susceptible, warfarin-resistant, and bromadiolone- and warfarin-resistant rats as determined with the BCR method are shown in pie diagrams. Sample sizes are indicated for resistance testing (numerator) and genetic typing (denominator). Circle sizes indicate the relative magnitude of each rat infestation in grams of bait taken as estimated by census baiting and are as follows: BK (5,917 g); WU (2,149 g); PS (1,830 g); and HT (971 g). No census baiting was done in population LH, but it is assumed to be very large because of the high frequency of infestation reported throughout this urban area.

(35), with some minor modifications as described (33), and adhered to strict humanitarian guidelines for animal husbandry and laboratory testing as recommended by the EPPO and as practiced by the BBA.

A relative size index for the farm populations WU, BK, PS, and HT was obtained by census baiting (36). During four days of nonpoisonous prebaiting, the maximum difference between supplied and recovered bait was noted as grams of bait taken and reflects consumption during the census session, which is a widely adopted measure of relative population size in rodent control programs (36). The bait was not depleted at any feeding station. We had no opportunity to estimate the relative size of population LH. The relative size indices of farm populations increased in the order HT, PS, WU, BK (Fig. 1).

Genetic Data Collection and Statistical Analysis. Primer pairs for amplification of 26 genetically mapped microsatellite markers (Fig. 4; ref. 31) were purchased from Research Genetics (Huntsville, AL). Mapping resources were as follows: http://waldo.wi.mit.edu/rat/public (at the Whitehead Institute/ MIT, Cambridge, MA); http://www.informatics.jax.org/rat/ ratMaps.shtml (at The Jackson Laboratory); http://ratmap.gen. gu.se (at the University of Gothenborg, Gothenborg, Sweden); and see references given in refs. 29 and 30. DNA preparation from liver and marker typing followed standard procedures (31) and were as previously used for linkage mapping of *Rw* (29, 30).

For each locus and population, gene diversity, H, was determined with the program FSTAT 1.2 (37). Hardy-Weinberg equilibrium (HWE) expectations for genotype frequencies, and their associated probabilities, were calculated with exact tests by using the Markov Chain method as implemented in the program package GENEPOP 3.1c (38, 39) at default settings (1,000 dememorization steps, 50 batches, and 1,000 iterations per batch). Score statistics (U tests) were used to specifically assess heterozygote deficiency and excess, and also by using the Markov Chain method at default settings. Probabilities associated with coefficients of linkage disequilibrium for all locus pairs and each population were estimated by using Fisher's exact test and the Markov Chain method using default settings as implemented in GENEPOP 3.1C (38, 40). Two loci were considered in LD when their associated P value was ≤ 0.01 (41).

We used an assignment test (42–44) to identify the chromosomal regions and individual loci that are most informative to discriminate among resistant and susceptible rats. For this, rats were grouped into three categories based on BCR test results without respect to their population affiliation as follows: (*i*) susceptible; (*ii*) warfarin-resistant and bromadiolone-susceptible; and (*iii*) warfarin- and bromadiolone-resistant (Fig. 1). A likelihood-based method that used a Bayesian algorithm implemented in the program GENECLASS (http://www.ensam.inra.fr/ URLB/geneclass/geneclass.html; ref. 42) was used to compute assignment probabilities. The "leave one out procedure" was used, and 10,000 simulated genotypes were created for likelihood calculations by randomly sampling alleles according to their frequencies in the pooled sample. The threshold for assignment was set to 0.1. The output of the program was used to determine the fraction of rats assigned correctly to resistance category (P_a) and to compute a likelihood ratio (45). The overall power of markers to discriminate among resistance categories based on their allelic and genotypic distributions was tested by using GENEPOP 3.1c at default settings (38, 46). For allelic differentiation, Fisher's exact test was used on contingency tables, and probabilities were computed after 1,000 randomizations by using the Markov Chain method. Genotypic differentiation was assessed in a similar fashion, but log likelihood ratios (G values) were computed after randomization of contingency tables.

Results and Discussion

As determined by the BCR method, the five rat populations sampled ranged from near complete resistance to full susceptibility to warfarin and bromadiolone, but no resistance to difenacoum was found (Fig. 1). Mean gene diversity systematically decreased with population resistance (Fig. 2A) whereas the number of loci that deviated from HWE increased with resistance level (Fig. 2B). In the nonresistant, presumably largest population LH, only 1 of 26 loci (3.8%) deviated from HWE whereas the corresponding values for populations WU, BK, PS, and HT were 11 (42.3%), 9 (34.6%), 3 (11.5%), and 7 (26.9%), respectively. Although suggestive, the relationship between departure from HWE and resistance level was not statistically significant. However, tests for HWE lack statistical power when expectations are calculated from gene frequencies measured after selection has already occurred (47). Testing specifically for the hypotheses of heterozygote deficiency and excess showed that all but one of 31 (96.7%) deviations were for heterozygote deficiency. Neither H nor the number of loci deviating from HWE predictions were significantly correlated with the size indices available for the populations WU, BK, PS, and HT (ANOVA, $P = 0.34$ and $P = 0.86$, respectively). Hence, selection for rodenticide-resistance is implicated as a major cause for this observed reduction in polymorphism.

In the Norway rat, resistance to the anticoagulant rodenticide warfarin is cited as an example of overdominant selection

$$y = 0.6 - 0.01x$$

$$y = 9.9 + 0.3x$$

$$\log(y) = -5.02 + 0.04x$$
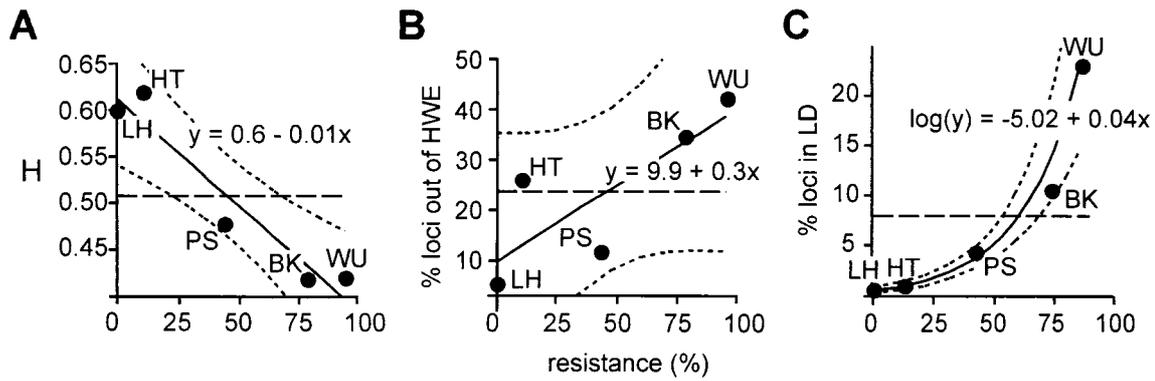
Fig. 2. Association of resistance with mean H, the fraction of loci that depart from HWE expectations, and LD. (*A*) Mean locus H versus resistance level (ANOVA, $F = 36.6$, $P < 0.01$, $R^2 = 0.92$). (*B*) Fraction of loci deviating from HWE versus resistance level (ANOVA, $F = 5.1$, $P = 0.1$, $R^2 = 0.63$). (*C*) Population LD ($P \leq 0.01$) versus resistance level (ANOVA, $F = 269.2$, $P < 0.001$, $R^2 = 0.99$). Populations and resistance levels as shown in Fig. 1. Lines are least-squares fit (solid), mean (dashed), and 95% confidence intervals (dotted).

(30–32), one of only a few mechanisms that maintain genetic variation in natural populations (2, 47). Under an overdominant selection model, heterozygotes have higher fitness and should therefore be found in excess of HWE expectations (47, 48). Moreover, overdominant selection maintains divergent haplotypes in populations (11), and, thus, gene diversity should increase with resistance. In contrast to these expectations, we found a reduction in H and increase of heterozygote deficiency with resistance level in genetic markers associated with *Rw*, suggesting that directional selection has occurred. One hypothesis that accounts for our results posits that the homozygous-resistant genotype may have an ephemeral advantage during pulses of intense anticoagulant treatment. Heterozygotes would increase in fitness and frequency over time as rodenticide levels in the environment decrease. Thus, overdominant selection only may be characteristic of populations exposed to persistent low levels of anticoagulant. Alternatively, as documented in insect pest species, continued use of ineffective pesticides can favor the evolution of modifier loci that ameliorate the epistatic costs initially associated with a resistance mutation (49), thereby increasing the relative fitness and frequency of the homozygous resistant genotype.

Intense natural selection is predicted to cause LD between loci physically linked to genes under selection. However, the size of the affected genomic window and its temporal persistence is uncertain in natural populations, given recombination and mutation (e.g., refs. 9 and 13). We found extensive LD between markers linked to *Rw* in the highly resistant populations WU and BK, occurring at 24% and 11% of all locus pair comparisons (Figs. 2*C* and 3). In contrast, only 0.5, 1, and 4% locus pairs were in LD in populations LH, HT, and PS, respectively (Figs. 2*C* and 3). The correlation between population LD and resistance level was highly significant (Fig. 2*C*). At drift-mutation-recombination equilibrium, LD and population size are expected to be inversely related (50). In accord with this prediction, the fully susceptible population, LH, which we assumed to be the largest, displayed the fewest locus pairs in LD (Figs. 2*C* and 3). However, the remaining four populations WU, BK, PS, and HT exhibited a pattern contrary to equilibrium predictions, and a nonsignificant relationship was found between population LD and their population size indices (ANOVA, $P = 0.92$). Consequently, because levels of population resistance rather than population size were correlated with LD, natural selection for anticoagulant poison-resistance is implicated as the predominant cause for population LD near the *Rw* locus.

The distribution of LD should be clustered closest to genes under selection, and, thus, this pattern may be used for fine-scale mapping (9, 12). In contrast, LD generated by drift and other population-specific variables is expected to be stochastic with respect to map location. To detect regions of clustered LD, we

	Arb18	Mit13	Mit2	R64	R65	R66	R67	R68	R69	R115	R130	R155	R193	R198	R202	R208	R216	R219	R286	R287	R289	R290	R292	R295	R360	R364
D1Arb18	-	BK		BK	BK				BK	BK								WU					BK			
D1Mit13		-		WU	WU	WU	WU	WU BK			WU	WU	WU	WU	WU			WU		BK			BK		WU	WU
D1Mit2			-	WU	WU						WU	WU	WU	WU											WU	
D1Rat64				-	WU BK	WU BK	WU BK	WU	BK		WU	WU BK	WU BK	WU BK	WU	WU		WU					WU		WU	BK
D1Rat65					-	WU BK	WU BK	WU	BK		WU BK	WU BK	WU BK	WU BK	WU		WU BK	WU	WU BK				WU		WU	WU BK
D1Rat66						-	WU	WU BK	BK		BK	WU BK	WU BK	WU BK	WU BK	WU		WU BK	WU BK		BK		WU		WU	
D1Rat67				LH			-	WU	BK		WU BK	WU BK	WU	WU BK			BK	WU BK	WU	BK	WU				WU	WU BK
D1Rat68				PS				-			WU	WU	WU		WU	WU BK		WU	WU		BK		WU	WU	WU	
D1Rat69				LH		PS			-						BK	WU BK	BK	BK								
D1Rat115										-																WU
D1Rat130					PS	HT					-	WU BK	WU BK	BK	WU		BK	WU BK	WU BK						WU	WU BK
D1Rat155												-	WU BK	WU	WU BK	WU	WU	WU	WU	BK	WU				WU	WU BK
D1Rat193				PS	HT						PS		-	WU BK	WU			WU	WU		WU				WU	WU BK
D1Rat198				HT			PS							-			BK	WU BK							WU	BK
D1Rat202		PS		HT	LH					LH			PS HT	PS	-			WU	WU BK		WU		WU		WU	WU
D1Rat208				LH							PS					-		WU	WU		WU			BK	WU	
D1Rat216												PS HT					-	WU BK								WU BK
D1Rat219				HT		PS	PS				PS	LH	PS		PS		PS	-	WU						WU	WU BK
D1Rat286	PS			HT		PS		PS		PS		HT	HT				PS		-		WU		WU		WU	WU BK
D1Rat287							LH			PS									PS	-				BK		BK
D1Rat289	.			HT																HT	-		BK		WU	
D1Rat290																					HT	-				
D1Rat292		PS		LH			LH		LH		PS				PS	PS			HT LH	LH			-	WU	WU	WU
D1Rat295			PS	PS			HT LH								HT				HT LH	LH				-	BK	
D1Rat360					PS									LH						PS					-	WU
D1Rat364					PS						PS			PS				PS HT			LH		PS			-

Fig. 3. Significant pairwise LD values between microsatellite markers. LD values significant at $P \leq 0.01$ (red) and at $P \leq 0.05$ (blue) are indicated. Populations WU and BK (above diagonal), PS, HT, and LH (below diagonal).
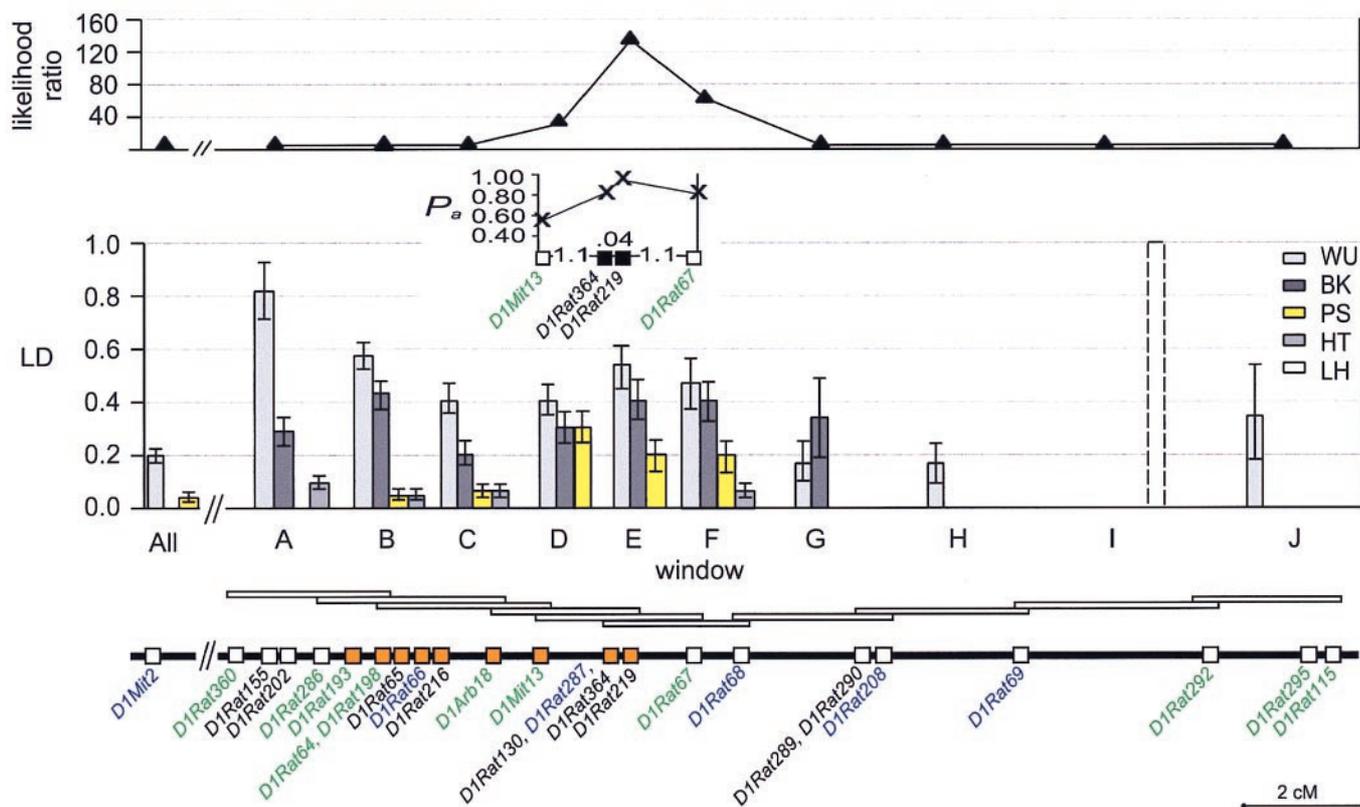
Fig. 4. Linkage-disequilibrium mapping of *Rw* by sliding window and likelihood ratio analysis. (*Lower*) For each population and windows A–J, the fraction of locus-pairs in LD ± one standard deviation was plotted on a 32-cM spanning segment of the MIT SHRSP × BN F_2-cross derived genomic map. The confidence in marker fine-scale placement decreases from blue to green to black (see http://waldo.wi.mit.edu/rat/public). Markers that fell within the 95% confidence interval about *Rw* (29) are shown in orange, and, for *D1Rat219* and *D1Rat364*, this was confirmed as described (29). (*Upper*) The average window likelihood ratio of assignment probabilities to resistance category, $P_{(correctly\ classified)}/P_{(incorrectly\ classified)}$. (*Insert*) The fraction of rats that were correctly classified (P_a) plotted versus marker position. Distances between markers are given in centimorgans. Markers that detected significant ($P < 0.01$) allelic and genotypic differentiation among resistance categories are shown in black. Note that, of the positionally unresolved markers *D1Rat364*, *D1Rat130*, and *D1Rat287*, the former was more informative than the latter two (not shown).

used an ≈3-cM-sized sliding window to scan the chromosome segment (9, 12). For each of the resulting 10 partially overlapping windows, A through J, the fraction of locus pairs in LD ($P ≤ 0.01$) was noted (Fig. 4). In the population with the highest resistance level, WU, 9 of 10 windows displayed LD. Moreover, 20% of comparisons of loci within the segment defined by windows A through J were in LD with *D1Mit2*, suggesting LD spanned the entire ≈32-cM segment, corresponding to ≈14% of rat chromosome 1. Similarly, in the highly resistant population, BK, the affected region was large and covered at least 11 cM. Population PS with an intermediate level of resistance had LD confined to windows D through F that define an ≈4.43-cM interval of which 2.24 cM (50.6%) overlap with the previously identified confidence interval about *Rw* (Fig. 4). In contrast, nonresistant population LH displayed LD only in window I, for which only a single locus pair was analyzed.

The physical extent of LD is well circumscribed in population PS, where three adjacent windows have high values of LD (Fig. 4). Additionally, LD was generally an order of magnitude lower in population HT than in the other populations with higher resistance levels and only about twice as high as background levels of LD reported for human populations (41). The more limited physical extent of LD in population PS suggests that populations with intermediate levels of resistance may be more appropriate for fine-scale mapping. According to a deterministic model of genetic hitchhiking, the size of the chromosome region affected by selection depends on the selection coefficient (e.g.,

refs. 5 and 6). Recent anticoagulant treatments differ among the rat populations (H.-J.P., unpublished data). Only warfarin was used during a single treatment in population PS whereas populations WU and BK received three and four treatments, respectively, that, in addition to warfarin, included the more potent compounds bromadiolone and difenacoum. Thus, a lower intensity of selection could explain the narrower chromosome window for LD found in population PS. Alternatively, a modifier locus affecting resistance to higher potency anticoagulants may be situated upstream of the *Rw* locus (Fig. 4).

The map position of *Rw* suggested by patterns of LD in population PS is strongly supported by a likelihood ratio test. For each window, we determined the ratio of the mean likelihood of a resistant rat to be correctly assigned over the likelihood of it being incorrectly assigned. The highest peak value was 135.9 in window E, with the next two highest likelihood ratios of 34.5 and 65.9 in adjoining windows D and F, respectively (Fig. 4). In contrast, the average likelihood ratio for the remaining windows was about two, corresponding to a random binomial chance of correct classification. Consequently, these results point to the 2.2-cM interval between *D1Mit13* and *D1Rat67* that is covered by window E as the location of informative markers most proximal to the warfarin-resistance gene.

For each marker, we tested for allelic and genotypic differentiation among resistance categories to identify those with the highest discriminating power (e.g., ref. 51). Only the two markers *D1Rat219* and *D1Rat364* allowed for a significant discrimination

of the three resistance categories (Fig. 4; $P < 0.01$), and, during an assignment test, *D1Rat219* distinguished susceptible from resistant rats in 91% of all cases (Fig. 4). The rate of decay of significance at the directly adjacent loci *D1Mit13* and *D1Rat67* suggests our mapping resolution may be on an $\approx$1-cM scale (Fig. 4). We sought additional and independent confirmation of the map location of *Rw* by comparative mapping between rat and mouse. We used our available congenic-resistant laboratory strain (29) to determine the linkage position of *D1Rat219* in relation to polymorphic gene-anchored loci whose map position was known in the rat and mouse (30). The resulting map position of *D1Rat219* corresponded to the interval 60–63 cM on mouse chromosome 7 (data not shown), which includes the linkage position of the *war* locus, the mouse ortholog to *Rw*, that has been previously inferred by its linkage to *frizzy* (25, 26).

Linkage disequilibrium between marker loci associated with fitness-related genes may be more frequent in natural populations than is assumed by population genetic models (2). First, a metaanalysis of 1,276 published selection coefficients documented that intense selection may be common in natural populations, and selection coefficients can attain values similar in magnitude to those of artificial breeding experiments (1). Roughly 18% of selection coefficients measured in unperturbed natural populations were higher than 0.6, which is similar in magnitude to selection coefficients associated with warfarin selection (22–24). Second, the natural environment generally is spatially and temporally heterogeneous, and, therefore, the relative fitness of genotypes is constantly changing (2). This flux in relative fitness is expected to lead to higher levels of LD than if the environment were constant (2). Finally, many experimentally or naturally perturbed populations are available for study in which selection coefficients tend to be higher than in undisturbed populations (1).

Here, we demonstrate that natural selection can cause linkage disequilibrium over a substantial genomic interval, justifying the search for the genetic signature of recent selective sweeps near other candidate genes, or across well mapped genomic segments. In organisms having well resolved genetic maps, whole-genome scans are now feasible (12), suggesting the possibility of genome-wide searches for major fitness-related genes. The mapping resolution achieved during our study of population linkage disequilibrium is comparable to that attained during our previous pedigree based linkage analyses (29, 30). Thus, by judicious choice of populations and species, "natural selection mapping" can provide a direct means to detect and map genes that are the object of natural selection.

1. Endler, J. A. (1985) *Natural Selection in the Wild* (Princeton Univ. Press, Princeton).
2. Gillespie, J. H. (1991) *The Causes of Molecular Evolution* (Oxford Univ. Press, New York).
3. Maynard-Smith, J. & Haigh, J. (1974) *Genet. Res.* **23,** 23–35.
4. Ohta, T. & Kimura, M. (1975) *Genet. Res.* **25,** 313–326.
5. Ewens, W. J. (1977) *Mathematical Population Genetics* (Springer, New York).
6. Thomson, G. (1977) *Genetics* **85,** 753–788.
7. Strobeck, C. (1983) *Genetics* **103,** 545–555.
8. Kaplan, N. L., Hudson, R. R. & Langley, C. H. (1989) *Genetics* **123,** 887–899.
9. Grote, M. N., Klitz, W. & Thomson, G. (1998) *Genetics* **150,** 1295–1307.
10. Stephan, W., Wiehe, T. & Lenz, M. W. (1992) *Theor. Popul. Biol.* **41,** 237–254.
11. Slatkin, M. (1995) *Mol. Biol. Evol.* **12,** 473–480.
12. Huttley, G. A., Smith, M. A., Carrington, M. & O'Brien, S. J. (1999) *Genetics* **152,** 1711–1722.
13. Wiehe, T. H. (1998) *Theor. Popul. Biol.* **53,** 272–283.
14. Lai, C., Lyman, R. F., Long, A. D., Langley, C. H. & MacKay, T. F. C. (1994) *Science* **266,** 1697–1702.
15. Long, A. D., Lyman, R. F., Langley, C. H. & MacKay, T. F. C. (1998) *Genetics* **149,** 999–1017.
16. Kreitman, M. & Hudson, R. R. (1991) *Genetics* **127,** 565–582.
17. Begun, D. J. & Aquadro, C. F. (1991) *Genetics* **129,** 1147–1158.
18. Wang, R.-L., Stec, A., Hey, J., Lukens, L. & Doebley, J. (1999) *Nature (London)* **398,** 236–239.
19. Paterson, S. (1998) *J. Hered.* **89,** 289–294.
20. Berry, A. J., Ajioka, J. W. & Kreitman, M. (1991) *Genetics* **129,** 1111–1117.
21. Rieseberg, L. H., Whitton, J. & Gardner, K. (1999) *Genetics* **152,** 713–727.
22. Greaves, J. H., Redfern, R., Ayres, P. B. & Gill, J. E. (1977) *Genet. Res.* **30,** 257–263.
23. Partridge, G. G. (1979) *Heredity* **43,** 239–246.
24. Bishop, J. A., Hartley, D. J. & Partridge, G. G. (1977) *Heredity* **39,** 389–398.
25. Greaves, J. H. & Ayres, P. B. (1969) *Nature (London)* **224,** 284.
26. Wallace, M. E. & MacSwiney, F. J. (1976) *J. Hyg.* **76,** 173–181.
27. Hildebrandt, E. F. & Suttie, J. W. (1982) *Biochemistry* **21,** 2406–2411.
28. Thijssen, H. H. W. (1995) *Pestic. Sci.* **43,** 73–78.
29. Kohn, M. H. & Pelz, H.-J. (1999) *Mamm. Genome* **10,** 696–698.
30. Kohn, M. H. & Pelz, H.-J. (2000) *Blood*, in press.
31. Jacob, H. J., Brown, D. M., Bunker, R. K., Daly, M. J., Dzau, V. J., Goodman, A., Koike, G., Kren, V., Kurtz, T., Lernmark, A., *et al.* (1995) *Nat. Genet.* **9,** 63–69.
32. Lund, M. (1985) *Acta Zool. Fennica.* **173,** 149–153.
33. Pelz, H.-J., Hänisch, D. & Lauenstein, G. (1995) *Pestic. Sci.* **43,** 61–67.
34. Martin, A. D., Steed, A. C., Redfern, R., Gill, J. E. & Huson, L. W. (1979) *Lab. Anim.* **13,** 209–214.
35. OEPP/EPPO (1995) *EPPO Bull.* **25,** 575–593.
36. Huson, L. W. (1980) *J. Hyg.* **84,** 341.
37. Goudet, J. (1993) *J. Hered.* **86,** 485–486.
38. Raymond, M. & Rousset, F. (1995) *J. Hered.* **86,** 248–249.
39. Rousset, F. & Raymond, M. (1995) *Genetics* **140,** 1413–1419.
40. Garnier-Gere, P. & Dillmann, C. (1992) *J. Hered.* **83,** 239.
41. Laan, M. & Pääbo, S. (1997) *Nat. Genet.* **17,** 435–438.
42. Cornuet, J. M., Piry, S., Luikard, G., Estoup, A. & Solignac, M. (1999) *Genetics* **153,** 1989–2000.
43. Paetkau, D., Calvert, W., Stirling, I. & Strobeck, C. (1995) *Mol. Ecol.* **4,** 347–354.
44. Rannala, B. & Mountain, J. L. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9197–9221.
45. Sokal, R. R. & Rohlf, F. J. (1995) *Biometry* (Freeman, New York).
46. Raymond, M. & Rousset, F. (1995) *Evolution (Lawrence, Kans.)* **49,** 1280–1283.
47. Hartl, D. L. & Clark, A. G. (1999) *Principles of Population Genetics* (Sinauer, Sunderland, MA).
48. Watterson, G. A. (1978) *Genetics* **88,** 405–417.
49. McKenzie, J. A. (1996) *Ecological and Evolutionary Consequences of Insecticide Resistance.* (R. G. Landes Company, Austin, TX).
50. Slatkin, M. (1994) *Genetics* **137,** 331–336.
51. Taylor, M. F. J., Shen, Y. & Kreitman, M. E. (1995) *Science* **270,** 1497–1499.

EVOLUTION