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Temporal Dynamics and Linkage Disequilibrium in Natural *Caenorhabditis elegans* Populations

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ABSTRACT

Caenorhabditis elegans is a major laboratory model system yet a newcomer to the field of population genetics, and relatively little is known of its biology in the wild. Recent studies of natural populations at a single time point revealed strong spatial population structure and suggested that these populations may be very dynamic. We have therefore studied several natural *C. elegans* populations over time and genotyped them at polymorphic microsatellite loci. While some populations appear to be genetically stable over the course of observation, others seem to go extinct, with full replacement of multilocus genotypes upon regrowth. The frequency of heterozygotes indicates that outcrossing occurs at a mean frequency of 1.7% and is variable between populations. However, in genetically stable populations, linkage disequilibrium between different chromosomes can be maintained over several years at a level much higher than expected from the heterozygote frequency. *C. elegans* seems to follow metapopulation dynamics, and the maintenance of linkage disequilibrium despite a low yet significant level of outcrossing suggests that selection may act against the progeny of outcrossings.

MOST population genetic studies infer evolutionary mechanisms of a population from a single time point. There are as yet few molecular studies of the same population over time (Viard *et al.* 1997; Guillemaud *et al.* 2003; Meunier *et al.* 2004; Charbonnel and Pemberton 2005; Trouvé *et al.* 2005). However, to obtain a direct picture of dynamic phenomena such as migration, recombination, selection, or population extinction and to detect variation over time, temporal surveys of populations are required. Here we present a temporal study of several natural populations of the nematode *Caenorhabditis elegans* over 3 years.

Beyond the fact that *C. elegans* is a major laboratory model organism with a fast generation time (3.5 days in standard laboratory conditions), an interesting feature for evolutionary biology is its peculiar mode of reproduction: *C. elegans* has two sexes, selfing XX hermaphrodites and facultative XO males that are able to mate with hermaphrodites. Males arise either spontaneously by rare nondisjunction of X chromosomes at meiosis (at a rate of ~0.1%; Hodgkin and Doniach 1997; Teotónio *et al.* 2006) or as progeny of hermaphrodites when mated with males (50% of the cross-progeny is male). This facultative outcrossing makes *C. elegans* an excellent system for studying the impact of outcrossing in a diploid organism.

Studies on natural populations of *C. elegans* have only recently begun. This species displays low overall levels of polymorphism (similar to humans, but 20-fold lower than *Drosophila melanogaster*) and displays only weak geographic structure at a worldwide scale (Koch *et al.* 2000; Denver *et al.* 2003; Barrière and Félix 2005; Cutter 2006, but see Haber *et al.* 2005). Selfing is clearly the predominant mode of reproduction in the wild, but outcrossing rate estimates range from 0.01% to 1–20%. The lower number (0.01%) was estimated from studies of linkage disequilibrium, either within local populations (Barrière and Félix 2005) or among a worldwide set of isolates (Cutter 2006). The higher range was estimated from measures of heterozygote frequencies in populations from France (1.3%; Barrière and Félix 2005) and Los Angeles (20%; Sivasundar and Hey 2005).

The habitat of *C. elegans* on ephemeral resources and its population genetic structure led to the suggestion that it may follow metapopulation dynamics (Barrière and Félix 2005; Sivasundar and Hey 2005), with populations frequently going extinct and habitats being recolonized through migration (Hanski 1999). However, this was suggested by indirect evidence, and a temporal study of local populations has so far been lacking. We have thus followed *C. elegans* populations in several locations over the span of 1–3 years, with the goal of identifying the population dynamics shaping its evolution. We found that some *C. elegans* populations were ephemeral and observed metapopulation dynamics, with extinction

A.B. dedicates this work to the memory of Daniel Lachaise, who had a decisive influence on his career.

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followed by recolonization by new genotypes. Surprisingly, in the largest and most stable population, genetic linkage between the same alleles persisted over 3 years despite a detectable rate of outcrossing, suggesting selection acting against the progeny of a recombination event. The observed metapopulation structure and the maintenance of linkage disequilibrium may explain the discrepancy between outcrossing rates measured at short and long timescales through heterozygote frequency and linkage disequilibrium, respectively. We finally discuss how the observed dynamics of natural *C. elegans* populations may influence the genetic and phenotypic evolution of this species.

MATERIALS AND METHODS

Sampling: The sampling procedure and most locations were described in Barrière and Félix (2005). New sampling locations include Obernai, Bas-Rhin, France (position: 48.46°N, 7.48°E), for leaf litter next to a vegetable garden in the middle of vineyards, and the Botanical Garden of the University of Lisbon (38.42°N, 9.12°W), for leaf and fruit litter below trees and in a compost heap. Samples were collected from September 2002 to January 2006 (details on sampling can be found in supplemental Table S1 at http://www.genetics.org/supplemental/).

Sampled material was spread on standard NGM plates seeded with *Escherichia coli* OP50. Worms were picked within 1 hr to 2 days after plating; the developmental stage was recorded, as described in Barrière and Félix (2006). On several occasions, six samples were taken a few centimeters apart in a single compost pile.

In most cases, individuals sampled from the soil were left to develop on the plates and self-progeny of hermaphrodites were harvested. One portion was frozen in glycerol and kept at −80° while the rest was lysed in worm lysis buffer (a digestion buffer commonly used to prepare DNA from *C. elegans*: 50 mm KCl, 10 mm Tris, pH 8.2, 2.5 mm $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin, 0.06% proteinase K), the lysate conserved at −20° and used for subsequent amplification. This procedure conserves the genotype of the collected individual, even when heterozygous (labeled "heterozygous" in Table 2). In other cases (labeled "inbred" in Table 2), isogenic strains were established by selfing for several generations (Barrière and Félix 2005), and possible heterozygosity was lost.

Microsatellite genotyping: Six microsatellite regions defined in Haber *et al.* (2005) were amplified by PCR. The forward primer was labeled with a fluorophore, either Hex or 6-Fam, and amplified fragments were run on a ABI 3100-avant system. Primers were *II-L*—f, AACAAAAATGTGGCAGGGAG; r, GGGTTACGGTAGTGGTACTGTAGG; *III-R*—f, GATGAATG GATATGACCGGC; r, TATCAGGCGTATCACCTCCC; *IV-L*—f, AAGATTTCTGCTAACGTGCTGA; r, AGTAACTTTGGTGCA GGTTCG; *V-L*—f, CGTTGGGACAGGATCTAGTTG; r, CGTT GGGACAGGATCTAGTTG; *X-R*—f, GCACACGCTTGAATGT CATAA; r, AAGAGCAGTAGCCGTTGTTGA.

For the *II-R* locus, we used a slightly different protocol, with the forward primer tailed with an M13 sequence and amplification conducted with a labeled M13 primer. Primers were f, CACGACGTTGTAAAACGACTTCTCATTGGAAAGTTGGGC; r, CAATACCGAGAAACGGATGAA.

When a putative heterozygote was found, the glycerol stock was thawed, revived, and individual worms were genotyped to check whether both alleles segregated. Microsatellite repeat numbers were deduced from PCR fragment length and comparison with the repeat number in N2.

For microsatellite locus *II-L* in samples from Le Perreux-sur-Marne, we had reproducibility problems and therefore excluded this locus from further analysis in this population. In several isolates from the Lis12-0705 sample, genotyping at locus *IV-L* revealed two different fragment sizes, corresponding to 35 and 44 repeats. This polymorphism did not segregate in the self-progeny as would be expected from a heterozygote at a single locus, and the progeny of a cross with N2 males displayed three allele sizes (that of N2 plus the two others from Lis12-0705), suggesting a duplication of this locus in Lis12-0705. Since one individual (LisbonP12D3, supplemental data set S1 at http://www.genetics.org/supplemental/) displayed only the 35-repeat allele, we considered this 35-repeat allele to be at the locus *IV-L* genotyped in other strains. Similarly, one individual from the Lis14-0705 sample displayed two fragment sizes, corresponding to 36 and 46 repeats; we considered the 36-repeat allele to be at the locus orthologous to *IV-L*.

Data analysis: The pairwise difference, the scaled mutation parameter Θ_{hom} and gene diversity H were calculated with Arlequin V. 3.01 (Excoffier *et al.* 2005) over all loci. Θ_{hom} derives from gene diversity by the relationship $H = \Theta_{hom}/\Theta_{hom} + 1$. Population structure, as measured by θ (Weir and Cockerham 1984), an estimator of F_{ST}, and 95% confidence intervals were calculated by bootstrap with FSTAT (Goudet 2001).

The best statistics to test for population differentiation with unbalanced samplings is not F_{ST} or its components, but the likelihood ratio G-statistic (Goudet *et al.* 1996). Differentiation between samples was tested in R (R Development Core Team 2003) with the package Hierfstat (Goudet 2005).

To calculate the inbreeding coefficient f (Weir and Cockerham 1984), an estimator of F_{IS}, we used the Genetic Data Analysis software (Lewis and Zaykin 2001). Its confidence interval was obtained by bootstrapping over loci. The selfing rate was calculated as $s = 2f/(1 + f)$.

Genotypes were obtained either on the pooled progeny of an individual sampled from the wild ("noninbred") or after inbreeding for a few generations in the laboratory by picking a single hermaphrodite individual ("inbred" strains, designated with a strain number preceded by JU, our laboratory strain designation at the Caenorhabditis Genetics Center). This feature is indicated for each sample in the second column of Table 2. For calculations involving comparisons between laboratory inbred and noninbred genotypes, we considered inbred strains that are homozygotes at all loci as noninbred: given the very high level of inbreeding witnessed in nonlaboratory-inbred populations, this can be considered a reasonably good approximation.

In the case of non-inbred genotypes, we deduced haplotypes from genotypes: in most cases, they were homozygous; for the five individuals that were heterozygous at two or more loci, we inferred the phase from other haplotypes found in the same sample.

The standardized index of association I_A^S (multilocus measure of linkage disequilibrium) and its significance (P-value) were calculated for the different samples with Lian v. 3.5 (Haubold and Hudson 2000), using the parametric test.

For the confidence intervals on linkage disequilibrium in Franconville, values of D' were calculated from data, and the expected genotype frequency was calculated on the basis of allele frequencies for gradually more distant values of D'. The concordance of these expected tables with the observed data was then calculated by a polynomial probability estimate. Pairwise comparisons between two time points were carried out using the same method, calculating the concordance of D' values between the two samples. The R function used is available from the authors upon request.

For estimations of generation times compatible with the observed linkage disequilibrium over time, the latter was

considered to decay according to $D'_{N+1} = (1 - r)D'_N$, N being the generation number, r being the recombination rate. At generation N, linkage disequilibrium would be $D'_N = (1 - r)^N D'_0$. From linkage disequilibrium, it is thus possible to calculate N as $N = \ln(D'_N/D'_0)/\ln(1 - r)$. However, in *C. elegans*, r is diminished by inbreeding and lower than the normal recombination rate. Therefore, the observed recombination rate is $r' = r(1 - F_{IS})$ (Nordborg 1997). Thus, $N = \ln(D'_N/D'_0)/\ln[1 - r(1 - F_{IS})]$.

Embryonic lethality: To check for possible incompatibility between genotypes, embryonic lethality and brood size were monitored in the F_2 progeny of interstrain crosses (JU360 males with JU361 hermaphrodites, and the reverse cross). F_1 hermaphrodites were picked at the L4 stage and transferred every 8 hr to a new plate until sperm exhaustion. Twenty-four hours after transfer, unhatched eggs were counted, and a further 24 hr later, larvae were counted and abnormal phenotypes were recorded. After they had finished laying, F_1 hermaphrodites were genotyped at the *II-R* locus to differentiate self- from cross-progeny. Self-progeny provided an internal control.

RESULTS

We first outline the sampling structure of the natural *C. elegans* populations that we followed, describing their habitat, developmental stage, and density fluctuations. We then turn to the microsatellite genotypes of isolated individuals, first analyzing the molecular diversity and heterozygote frequency in each locality and the spatial structure at different scales. Finally, we analyze the temporal dynamics of these populations and the dynamics of linkage disequilibrium.

Habitat and population density: *Samplings:* We sampled *C. elegans* in different locations in France and Portugal, including those of our previous study (Barrière and Félix 2005) (Figure 1A). In several instances, samples were collected at different points within a location to probe for population structure at a small spatial scale (Figure 1, B–D). In addition to compost heaps, we sampled rotting fruits in the same gardens and in the Botanical Garden in Lisbon.

The localities of our previous study had been sampled either in 2002 or in 2004 (Barrière and Félix 2005). Among those, the Franconville, Le Perreux, Le Blanc, and Hermanville compost heaps were newly sampled at frequent intervals until January 2006. Only the two former yielded *C. elegans* every time (Franconville) or at most time points (Le Perreux). For the two latter, we could find *C. elegans* again only once or twice in 2005. We also resampled in 2005 the other localities of our previous study (Merlet, Primel, Sainte-Barbe). Each sample was named using the first letters of the location followed by the month and year of sampling (*e.g.*, Fra-1102 for the November 2002 sampling of Franconville) (Table 1 and supplemental Table S1 at http://www.genetics.org/supplemental/). Given the very high selfing rate, a population is difficult to define for *C. elegans*. For the sake of simplicity, we herein refer to all *C. elegans* individuals from a single location (*e.g.*, a single compost pile) as a population.

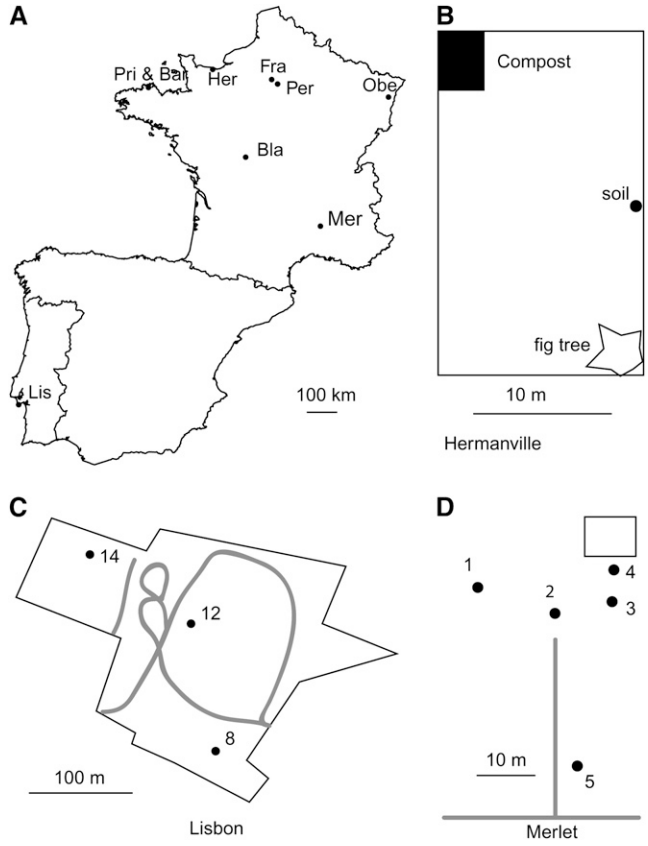

Figure 1.—Sampling locations. (A) Map of sampling locations in mainland France and Portugal. (B) Sketch of the Hermanville sampling location. (C) Sketch of the Lisbon sampling location. (D) Sketch of the Merlet sampling location. Scales are indicated for each map.

Habitat and developmental stage: Our sampling procedure allowed us to isolate all *C. elegans* individuals from a sample, most of them within a few hours (Barrière and Félix 2006). In addition to previously described habitats (compost heaps, soil, and snails), we found *C. elegans* in rotting fruits fallen below trees: apples, figs, tomatoes, plums, pears, and fruits from a Ficus tree and from an unidentified tree in the Lisbon Botanical Garden. Stages other than dauer were found mainly in fresh compost and decaying fruits in samples Bla-1105, HerF-1005, and Lis12-0705 (Table 1).

Density at a given time: Overall, density was higher in fresh compost and decomposing fruits than in older compost or soil. The highest density was found in fresh compost in Pri-0805 (21 individuals/gram; supplemental Table S1 at http://www.genetics.org/supplemental/). Within the compost heaps where several samples were taken at the same time, strong variations in *C. elegans* concentration could sometimes be found at a small scale (supplemental Table S1 and supplemental Figure S1).

Density over time: Given the large variance observed between samples collected on the same day, it may be hazardous to draw a strong conclusion on temporal dynamics using time points when a single sample was collected.

TABLE 1

Sampling of *C. elegans* populations

Location	Sample	Date	Mass (in g)	Individuals	Density	Nature	Stages/remarks
Sainte Barbe	Bar-1004	October 3, 2004	15	98	6.53	Compost	Described in Barrière and Félix (2005)
	Bar-0805	August 27, 2005	27.3	38	1.39	Compost	2 L4
		August 27, 2005		4		Isopods	3 dauer
Primel	Pri-1004	October 3, 2004	15	32	2.13	Compost and isopods	Described in Barrière and Félix (2005)
	Pri-0805	August 27, 2005	52	161	3.09	Compost	13 dauer, 2 L3, 1 L4, 1 adult
		August 27, 2005		7		Isopods	6 dauer
		August 27, 2005				Decomposing *Helix aspersa* snail	
Le Blanc	Bla-0802	August 25, 2002		13		Compost	Described in Barrière and Félix (2005)
	Bla-0305	March 28, 2005	35	6	0.17	Compost	3 L3
	Bla-0605	June 12, 2005	115	1		Compost	
	Bla-0805	August 15, 2005	131.5	0		Compost	
	Bla-1105	November 1, 2005	98	23	0.23	Compost	3 dauer, 3 L3, 8 L4, 9 adults
Hermanville	HerC-0902	September 22, 2002		12		Compost	Described in Barrière and Félix (2005)
	HerC-1105	November 8, 2005	20	38	1.9	Compost	Mixed L3-L4-adults
	HerF-1005	November 1, 2005		47		Figs	28 dauer, 6 L4, 10 adults
	HerF-1105	November 8, 2005		12		Figs	High density
	HerD-1105	November 8, 2005		1		Recovered from a cleaned fig placed as a fly bait above compost	
	HerS-0603	June 1, 2003		1		Caught in soil, near a snail	
Franconville	Fra-1102	September 16, 2002		12		Compost	Described in Barrière and Félix (2005)
	Fra-1004	October 6, 2004	13	130	10	Compost	Described in Barrière and Félix (2005)
	FraS-1004	October 6, 2004		4		Snails next to compost	Described in Barrière and Félix (2005)
	Fra-1204	December 8, 2004	9.5	43	4.53	Compost	Some non-dauer
	Fra-0205	February 14, 2005	31	40	1.29	Compost	33 dauer
	Fra-0405	April 6, 2005	34	11	0.32	Compost	1 L3, not all picked
	Fra-0505	May 20, 2005	42	14	0.33	Compost	
	Fra-0605	June 7, 2005	42	184	4.38	Compost	
	Fra-0705	July 19, 2005	59	64	1.08	Compost	55 dauer
	Fra-0805	August 1, 2005	128	86		Compost	All dauer, 1 male
	Fra-0905	September 23, 2005	125	29		Compost	19 dauer, 1 L3, 7 L4, 1 adult
	Fra-0106	January 7, 2006	99	57	0.57	Compost	43 dauer
Lisbon	Lis8-0705	July 10, 2005		45		Dates and leaf litter	1 L2, 38 dauer, 4 L3, 2 L4
	Lis12-0705	July 10, 2005		77		Figs from *Ficus isophlebia* and associated litter	3 L1, 8 L2, 55 dauer, 1 L3, 1 L4, 8 adults
	Lis14-0705	July 10, 2005		17		Compost	4 dauer, 2 L4, 1 adult
Merlet	Mer1-0902	September 8, 2002		13		Soil below hackberry tree	Described in Barrière and Félix (2005)
	Mer2-0902	September 8, 2002		4		Snails on mulberry tree	Described in Barrière and Félix (2005)
	Mer3-0902	September 8, 2002		4		Compost	Described in Barrière and Félix (2005)
	Mer1-1005	October 23, 2005	49	12	0.24	Soil below hackberry tree	10 dauer
		October 23, 2005	—	9		Pomatias snails	
	Mer3-1005	October 23, 2005	29	1	0.03	Compost	

(*continued*)

TABLE 1

(Continued)

Location	Sample	Date	Mass (in g)	Individuals	Density	Nature	Stages/remarks
	Mer4-1005	October 23, 2005		1		Figs	
	Mer5-1005	October 23, 2005		9		Apples	1 L2, 4 dauer, 3 L4, 1 adult
Obernai	Obe-1005	October 3, 2005		29		Fruit in orchard	1 L1, 8 dauer, 4 L4, 4 adults
Le Perreux	Per-0704	July 7, 2004		6		Compost	Described in Barrière and Félix (2005)
	Per-1004	October 5, 2004	17	36	2.12	Compost	Described in Barrière and Félix (2005)
	Per-1204	December 14, 2004	30	10	0.33	Compost	1 L4, 1 adult
	Per-0205	February 7, 2005	79	13	0.16	Compost	9 dauer
	Per-0405	April 5, 2005	62	0		Compost	
	Per-0605	June 8, 2005	68	6	0.09	Compost	6 dauer
	Per-0705	July 13, 2005	20	10	0.5	Compost	
	Per-0905	September 26, 2005	102	45	0.44	Compost	All dauer
	Per-101205	December 10, 2005	79	2	0.03	Compost	2 dauer
	Per-1205	December 19, 2005	80	13	0.16	Compost	13 dauer

"Location," name of sampling location; "Sample," name of sample; "Date," date of sampling; "Mass," mass of sample (in grams); "Individuals," number of *C. elegans* individuals recovered; "Density," density of sample (individuals per gram); "Nature," nature of sample and habitat; "Stages/remarks," occurrence of different developmental stages when we were able to determine them, and remarks about the sample. The four larval stages are indicated as L1–L4. See supplemental Table S1 at http://www.genetics.org/supplemental/ for more details, especially of subsamples.

However, in Franconville and Le Perreux, where samples were taken most regularly, densities seemed to decrease consistently during winter and spring, when food was presumably scarce and temperatures low (supplemental Figure S1 at http://www.genetics.org/supplemental/). Density was consistently lower in Le Perreux than in Franconville and we could not find any *C. elegans* in Le Perreux in April 2005 (Table 1). In addition, at most time points we could not find any *C. elegans* in Le Blanc and Hermanville compost heaps, even after processing copious amounts of compost sampled at different points in the heap (however, on several occasions we found *C. briggsae*, a relative of *C. elegans*).

Molecular diversity and outcrossing rate: A random subset of the isolated animals was genotyped at six microsatellite loci, named *II-R*, *II-L*, *III-R*, *IV-L*, *V-L*, and *X-R* according to their chromosomal arm position (see materials and methods; Haber *et al.* 2005). Haplotypes are identified by the location name followed by a letter (Figure 4 and supplemental Figure S2 at http://www.genetics.org/supplemental/).

Molecular diversity: Diversity, as measured either by pairwise differences in microsatellite haplotype or by gene diversity over the six loci, showed striking variations among samples (Table 2). Whereas some samples (Obe-1005, Lis12-0705, all Fra, all HerC) were very polymorphic, others (Per-0205, Per-0604, Per-0905, Mer1-0902, Mer2-0902, Mer3-0902, HerF-1105) were monomorphic. The low-density compost heap in Le Perreux was found to be less polymorphic than the high-density compost heap in Franconville.

Heterozygote frequency and outcrossing rate: Of the 540 individuals that we genotyped without prior selfing (see materials and methods), we found 10 heterozygotes, 5 of which were found in the Bla-1105 sample (Table 2) (this does not include the already described heterozygotes in samples Per-1004 and Fra-1004; Barrière and Félix 2005). We calculated the equilibrium inbreeding coefficient *f*, an estimator of the inbreeding coefficient F_{IS}, from which we deduced the selfing rate *s* and outcrossing rate $(1 - s)$ (see materials and methods). Estimated outcrossing rates ranged from 0 to 7.6% (Bla-1105) (Table 2). Calculated over all diploid genotypes, the mean outcrossing rate of the different populations was 1.7% (C.I.: 1.1–2.5%). This is very consistent with our previous results (1.3%) (Barrière and Félix 2005). In addition, the present results suggest that outcrossing rates vary among populations and provide an example of a population (Bla-1105) where outcrossing rates are significantly higher than in other samples and closer to those found in Sivasundar and Hey (2005) (~20%).

Male frequency and genotype: We found 2 males of 993 individuals (samples Fra-0805 and Obe-1005). Together with our previous samplings (Barrière and Félix 2005), we obtained a total of 4 males from 2269 individuals, yielding an overall male frequency of 0.18%.

TABLE 2

Molecular diversity and outcrossing rates in the sampled *C. elegans* populations

			Genetic diversity					Outcrossing					
Sample	Inbred?	*N*	Pairwise difference	*H*	SD	Θ_{hom}	SD	No. of heterozygotes	Observed heterozygosity	*f*	C.I.	*s*	C.I.
Bar-1004	h	15	1.251	0.699	0.084	1.774	0.739	0	0.000	1.000		1.000	
Bar-0805	h	14	2.152	0.605	0.052	1.151	0.257	1	0.067	0.940	(0.877–1)	0.969	(0.934–1)
Bla-0802	i	13	0.282	0.271	0.099	0.277	0.138						
Bla-1105	h	24	3.437	0.832	0.046	4.014	1.432	5	0.208	0.858	(0.819–0.901)	0.924	(0.901–0.948)
Fra-1102	i	12	1.621	0.507	0.093	0.769	0.289						
Fra-1004	h	12	1.522	0.754	0.058	2.377	0.793	0	0.000	1.000		1.000	
Fra-1204	i	12	1.621	0.797	0.067	3.110	1.394						
Fra-0205	i	12	1.712	0.609	0.087	1.171	0.438						
Fra-0405	h	6	1.818	0.727	0.109	2.054	1.190	0	0.000	1.000		1.000	
Fra-0505	h	12	1.779	0.757	0.060	2.427	0.846	2	0.167	0.865	(0.755–1)	0.927	(0.86–1)
Fra-0605	h	82	1.003	0.533	0.045	0.852	0.156	1	0.012	0.988	(0.969–1)	0.994	(0.984–1)
Fra-0705	i	12	1.152	0.522	0.099	0.815	0.327						
Fra-0905	h	18	1.594	0.679	0.024	1.613	0.185	0	0.000	1.000		1.000	
Fra-0106	h	57	0.896	0.504	0.051	0.760	0.158	0	0.000	1.000		1.000	
HerC-0902	i	12	3.758	0.812	0.040	3.436	0.979						
HerC-1105	h	19	2.504	0.762	0.068	2.502	1.002	0	0.000	1.000		1.000	
HerF-1005	h	45	0.261	0.170	0.053	0.153	0.058	0	0.000	1.000		1.000	
HerF-1105	h	11		0.000				0	0.000				
Lis8-0705	h	9	1.098	0.680	0.108	1.616	0.839	0	0.000	1.000			
Lis12-0705	h	20	3.103	0.836	0.042	4.125	1.386	0	0.000	1.000			
Lis14-0705	h	9	0.993	0.392	0.133	0.480	0.268	0	0.000	1.000		1.000	
Mer1-0902	i	11		0.000									
Mer1-1005	h	18	0.324	0.210	0.088	0.198	0.104	0	0.000	1.000		1.000	
Mer2-0902	i	4		0.000									
Mer3-0902	i	4		0.000									
Mer3-1005	h	1		0.000				0	0.000				
Mer4-1005	h	1		0.000				0	0.000				
Mer5-1005	h	4	2.714	0.857	0.082	4.935	3.621	0	0.000	1.000		1.000	
Obe-1005	h	18	0.979	0.884	0.034	6.428	2.350	1	0.056	0.973	(0.92–1)	0.986	(0.958–1)
Per-0604	h	10		0.000				0	0.000	1.000		1.000	
Per-1004	h	12	0.464	0.304	0.115	0.326	0.176	0	0.000	1.000		1.000	
Per-1204	i	9	2.028	0.758	0.077	2.440	1.100						
Per-0205	i	12		0.000									
Per-0605	h	6	0.303	0.303	0.148	0.324	0.226	0	0.000	1.000		1.000	
Per-0705	i	10	0.556	0.779	0.061	2.766	1.056						
Per-0905	h	44		0.000				0	0.000				
Per-1205	h	13	1.329	0.394	0.107	0.484	0.218	0	0.000	1.000		1.000	
Pri-1004	h	35	1.559	0.601	0.069	1.134	0.335	0	0.000	1.000		1.000	
Pri-0805	h	11	2.615	0.537	0.090	0.867	0.320	0	0.000	1.000		1.000	

"Inbred?," whether individual genotypes were scored after laboratory inbreeding ("i," inbred strains) or without ("h," potentially heterozygous); "*N*," number of genotyped individuals; "Pairwise difference," mean number of different pairwise microsatellite loci; "*H*," gene diversity (calculated with Arlequin; Excoffier *et al.* 2005) and its standard deviation (SD); "Θ_{hom}," Θ-parameter calculated after homozygosity and its standard deviation; *f*, equilibrium inbreeding coefficient (estimator of F_{IS} after Weir and Cockerham 1984, calculated with GDA; Lewis and Zaykin 2001) and its 95% confidence interval (C.I.); *s*, selfing rate calculated after *f* and its 95% confidence interval.

TABLE 3

Spatial structure of genetic differentiation at different scales

Sample	*P*-value	θ	C.I.
Structure within one compost pile			
Fra-0106	0.990		
Fra-0905	0.650		
Per-1205	0.792		
Mer1-1005 (snails-soil)	0.792		
Pri-0805 (snails-compost)	0.792		
Fra-1004(snails-compost)	0.133		
Bar-0805 (isopods-compost)	0.420		
Within one garden			
Lisbon	<0.001	0.366	0.189–0.504
Hermanville	<0.001	0.255	0.207–0.295
Merlet 05	<0.001	0.776	0.598–0.886
Primel–Sainte-Barbe 04	<0.001	0.591	0.453–0.664
Primel–Sainte-Barbe 05	<0.001	0.443	0.270–0.670
Merlet 02	<0.001	1.000	1.000–1.000
Large scale			
All locations (Jul–Nov 05)	<0.001	0.532	0.445–0.605

"*P*-value," *P*-value of differentiation test after Bonferroni correction. Values <0.05 indicate a significant spatial structure; "θ," estimator of F_{ST} after Weir and Cockerham (1984) with its C.I.

We placed each of the two males with *unc-119* mutant hermaphrodites (bearing a recessive mutation resulting in uncoordinated movements) and scored non-uncoordinated progeny. The male from the Fra-0805 sample sired no progeny, while the cross with the male from the Obe-1005 sample succeeded. Four of its progeny were genotyped at six loci and were found to be identical, indicating that the male was a homozygote at these six loci. The male may thus have been either a spontaneous male resulting from X chromosome nondisjunction or a male resulting from biparental inbreeding (mating among identical genotypes).

Structure at different spatial scales: *Within a single compost heap:* To determine whether the compost piles that we followed most closely (Franconville and Le Perreux) could each be considered homogenous, we measured spatial differentiation in genotype among samples from six different points within the pile (separated by 10–50 cm). In the three cases in which enough individuals were isolated from the different samples and showed polymorphism, no significant genetic structure was found (Table 3). Thus, even though there were strong differences in density within a compost heap, we found no evidence of genetic structure within a heap. We therefore considered nematodes from a given compost pile to be genetically homogenous (what we call a population).

When comparing the genotypes of *C. elegans* individuals found in soil in Merlet or in compost in Franconville, Primel, and Sainte-Barbe to those found in invertebrates (snails or isopods) sampled from the same habitat, no significant genotypic substructure was found (Table 3).

Within a garden: structure at the scale of tens of meters: Strong and highly significant genetic structure at a given time point was found at the next spatial scale when comparing samples within the same garden in Hermanville (10–20 m), Merlet (10–100 m), Lisbon (100–300 m), and between the Primel and Sainte-Barbe compost heaps (1 km) (Table 3). This was in agreement with the very strong structure observed within the Merlet location in 2002 (Barrière and Félix 2005).

Large-scale structure and haplotype sharing: At the global scale (100–1000 km scale), differentiation was significant between locations (Table 3), confirming our previous results ($F_{ST} = 0.78$; Barrière and Félix 2005). Except for two neighboring Primel and Sainte-Barbe locations (1 km apart), which share one haplotype at different time points (see below), only three instances of haplotype-sharing between locations were found (Bla-B and Her-A, Bla-N and Her-K, Bla-I and Mer-E).

In summary, *C. elegans* appeared to show no structure at a very small scale of a few centimeters, and very strong structure at scales >10 m; at a larger scale of hundreds of kilometers, structure appears weaker again, as previously observed (Barrière and Félix 2005; Cutter 2006).

Temporal dynamics of genotypes: The main aim of our sampling was to analyze population dynamics over time. Genetic diversity fluctuated over time for a given locality, especially in Le Perreux, where it reached zero at several time points (Table 2). Most strikingly, allele frequencies varied dramatically, sometimes even between samples collected only 6 weeks apart. For the two localities followed most closely, Franconville and Le Perreux, we conducted pairwise comparisons of multilocus genotypes of consecutive samples. In Franconville, a single pair of consecutive samples was significantly differentiated, whereas in Le Perreux, several pairs were significantly differentiated (asterisks in Figures 2B and 3B; Table 4).

In Franconville, where *C. elegans* could be isolated at all time points, the same major alleles at the three polymorphic loci (*II-R*, *II-L*, and *III-R*) were conserved over time (Figure 2A). Allele frequencies fluctuated, but overall the population was rather stable.

By comparison, Le Perreux showed much stronger variations; after the density decreased in late winter 2004, and sample Per-0405 yielded no *C. elegans*, new alleles were found at loci *II-R* and *X-R* in June 2005 (Figure 3A). In July 2005, another replacement took place (at loci *X-R* and *V-L*). In September 2005, the alleles and haplotypes found before April returned. Alleles found in June 2005 were found again in a single individual in December 2005 (supplemental Figure S2 at http://www.genetics.org/supplemental/, haplotype Per-G). This strongly suggested an extinction of the population, followed by recolonization by new genotypes.

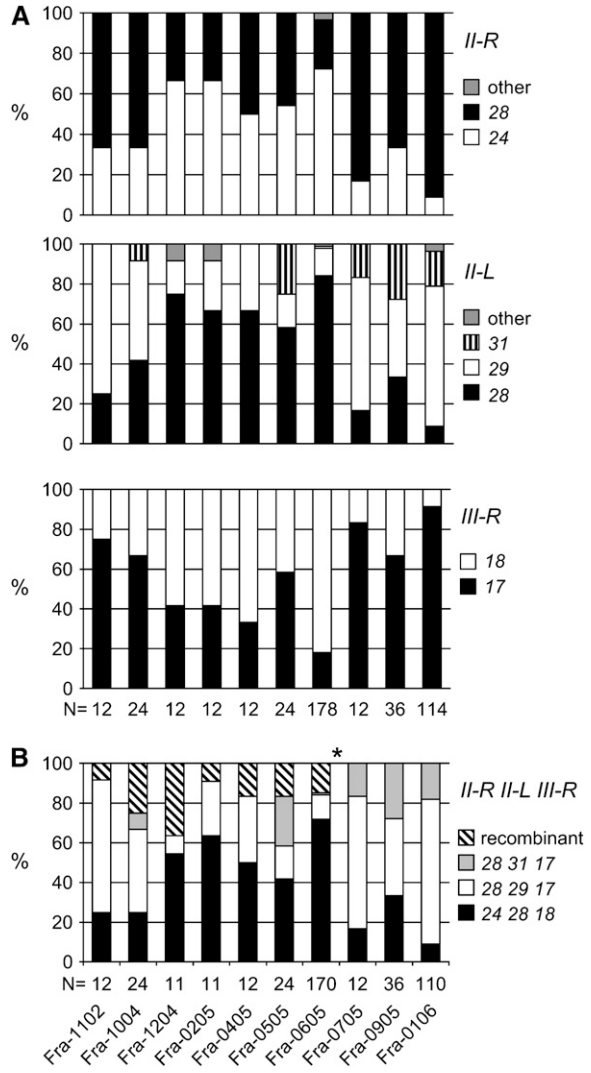

Figure 2.—Temporal survey of allele and multilocus genotype frequencies in the Franconville population. (A) Allele frequencies at loci *II-R*, *II-L*, and *III-R* (the most polymorphic loci in this population) over time in the Franconville population. The repeat number is indicated for each locus on the right. The number of genotyped individuals (*N*) is indicated below each time point (horizontal axis). (B) Frequencies of multilocus genotypes for the major alleles at the same three loci. Individuals showing a recombination between the major genotypes are indicated as recombinants. Rare haplotypes (<2% when considering all time points) were removed from this analysis. For more detailed data, see supplemental Figure S2G at http://www.genetics.org/supplemental/.

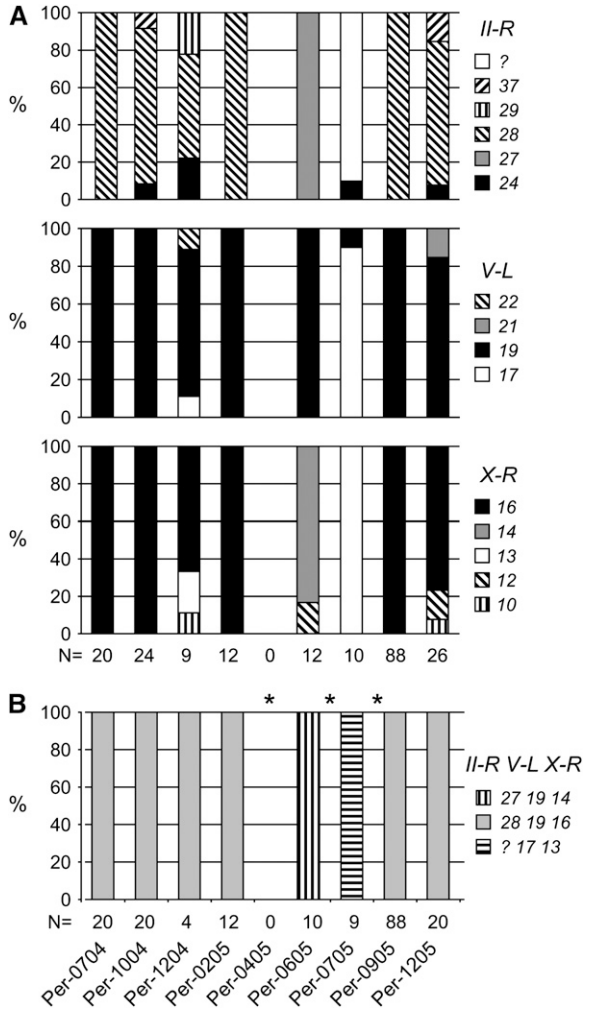

Figure 3.—Temporal survey of allele and multilocus genotype frequencies in the Le Perreux-sur-Marne population. (A) Allele frequencies at loci *II-R*, *V-L*, and *X-R* in the Le Perreux population, displayed as in Figure 2. For locus *II-R* in sample Per-0705, amplification repeatedly failed for several individuals (indicated as "?"). (B) Frequencies of multilocus genotypes for the major alleles at three loci in Le Perreux. No evidence of recombination between the three major genotypes was found. Rare haplotypes (<2% when considering all time points) were removed. Asterisks indicate significant differentiation between consecutive samples. For more detailed data, see supplemental Figure S2F at http://www.genetics.org/supplemental/.

For the five other localities, we analyzed samples separated by 1–3 years. Merlet 1 showed no significant differentiation over 3 years, the same major haplotype Mer-E being present in both samples (Table 4, supplemental Figure S2D). Hermanville showed a marginally nonsignificant differentiation after Bonferroni correction, with a single minor haplotype (Her-D) being shared between the two time points (Table 4, supplemental Figure S2B. The three other localities (Le Blanc, Primel, and Sainte-Barbe) showed very significant temporal differentiation (Table 4). Le Blanc witnessed an increase in genetic diversity that suggested an input from migration (several new alleles at several loci; Figure 4): the mutation rate was measured for locus *II-L* at 1.8×10^{-4} and for locus *IV-L* at 2.7×10^{-4}/generation (Frisse 1999) and these rates are too low to account for the increase in diversity observed in this locality. The Primel/Sainte-Barbe sampling locations (1 km apart) each showed strong temporal differentiation. As noted earlier, the major haplotype (PriBar-B; Figure 4) of Pri-1004 was found at a high frequency in sample Bar-0805 while it was absent in samples Pri-0805 and Bar-1004, an indication

TABLE 4

Temporal structure of genetic differentiation

Samples	*P*-value	θ	C.I.
HerC-1102–HerC-1105	0.056	0.159	0.080–0.216
Mer1-0902–Mer1-1005	1		
HerF-1005–HerF-1105	1		
Pri-1004–Pri-0805	<0.001	0.494	0.253–0.639
Bar-1004–Bar-0805	0.032	0.254	0.095–0.382
Between Fra samples			
Fra-1102–Fra-1004	0.662		
Fra-1004–Fra-1204	0.963		
Fra-1204–Fra-0205	1		
Fra-0205–Fra-0405	1		
Fra-0405–Fra-0505	1		
Fra-0505–Fra-0605	0.275		
Fra-0605–Fra-0705	<0.001	0.505	0.430–0.546
Fra-0705–Fra-0905	1		
Fra-0905–Fra-0106	0.275		
Between Per samples			
Per-0604–Per-1004	1		
Per-1004–Per-1204	0.963		
Per-1204–Per-0205	0.065		
Per-0205–Per-0605	<0.001	0.965	0.891–0.979
Per-0605–Per-0705	<0.001	0.842	0.736–0.931
Per-0705–Per-0905	<0.001	0.956	0.869–0.993
Per-0905–Per-1205	0.108		

"*P*-value," *P*-value of differentiation test after Bonferroni correction; "θ," estimator of F_{ST} after Weir and Cockerham (1984) with its C.I.

that this temporal differentiation was in part due to migratory input.

Thus, natural populations of *C. elegans* can display dramatic changes in allele frequencies over short periods of time. In at least two localities (Le Perreux and Le Blanc), these variations were associated with density decline and subsequent recolonization events. In contrast, in two other locations with larger *C. elegans* populations (Franconville, Merlet 1), stable genotypes were maintained over several years.

Strikingly, alleles at different loci remained associated over time within a locality, suggesting little effective outcrossing (Figure 2B and supplemental Figure S2 at http://www.genetics.org/supplemental/). We therefore investigated the dynamics of linkage disequilibrium between loci.

Linkage disequilibrium: *Multilocus linkage disequilibrium within a sample:* The level of linkage disequilibrium between all loci was very high and significant for all polymorphic samples except Fra-1204 (supplemental Table S3 at http://www.genetics.org/supplemental/). This is consistent with our previous observations based on AFLP data (Barrière and Félix 2005).

Linkage disequilibrium over time: A striking fact was the maintenance of very strong linkage disequilibrium between the same alleles for loci on different chromosomes over 3 years in Franconville. Indeed, the same two major multilocus genotypes in linkage disequilibrium were found throughout the 3 years, with very few recombinant genotypes (Figure 2B).

We looked for any evidence of decay of linkage disequilibrium in this locality. We calculated the linkage disequilibrium D between loci *II-R* and *III-R*, the two biallelic loci, and the associated D' (scaled by the maximum linkage-disequilibrium level possible within the sample). We chose to work with the classical coefficient of linkage disequilibrium D because it is a simple statistic, whose decay equation is trivial (see below). Linkage disequilibrium levels were indeed very high and remained high for >3 years (Figure 5). The sign of D' was the same in all samples, indicating that the polarity of linkage disequilibrium was conserved. The D' measure for the Fra-1204 sample was significantly lower than for later samples (comparing Fra-1204 with Fra-0106, the *P*-value $= 1.69 \times 10^{-5}$), which would indicate an increase (not a decrease!) in linkage disequilibrium over time. If we considered sample Fra-1204 as an anomaly and discarded it, the confidence intervals of D' for all samples were compatible. In any case, high linkage disequilibrium between the same alleles was maintained over 3 years. Given the density observed (see Table 1), the census size at the scale of the compost pile must be on the order of tens of thousands; therefore, drift alone cannot explain the absence of increase in recombinant frequency.

The outcrossing rate in Franconville was typical of results from all populations (0.9% over all time points), so the maintenance of linkage between alleles over such a long period of time was particularly puzzling. To test whether selfing alone could explain this level of linkage disequilibrium, we calculated the number of generations that would be compatible with the estimated outcrossing rate and the maintenance of linkage disequilibrium. The maximum decay of linkage disequilibrium compatible with our data would be from $D'_0 = 1$ in generation *0* (upper bound of confidence interval for sample Fra-1102) down to $D'_N = 0.947$ in generation N (lower bound for sample Fra-0106). Using these numbers, we estimated (see materials and methods) the maximum number of generations N_{max} as 6.0 generations over 38 months (1 every 6.3 months). With the average outcrossing rate over all our samples, N_{max} would be 4.2 generations (1 every 9 months). These values are hardly compatible with the known generation time and life expectancy in *C. elegans*, which in standard laboratory conditions are 3.5 days and 2 weeks, respectively. The generation time could be much longer in the wild, depending on temperature, food availability, occurrence of diapause, etc. However, it appears unlikely that *C. elegans* reproduces with an average of two generations per year. Therefore, the maintenance of linkage between loci must be explained by other factors.

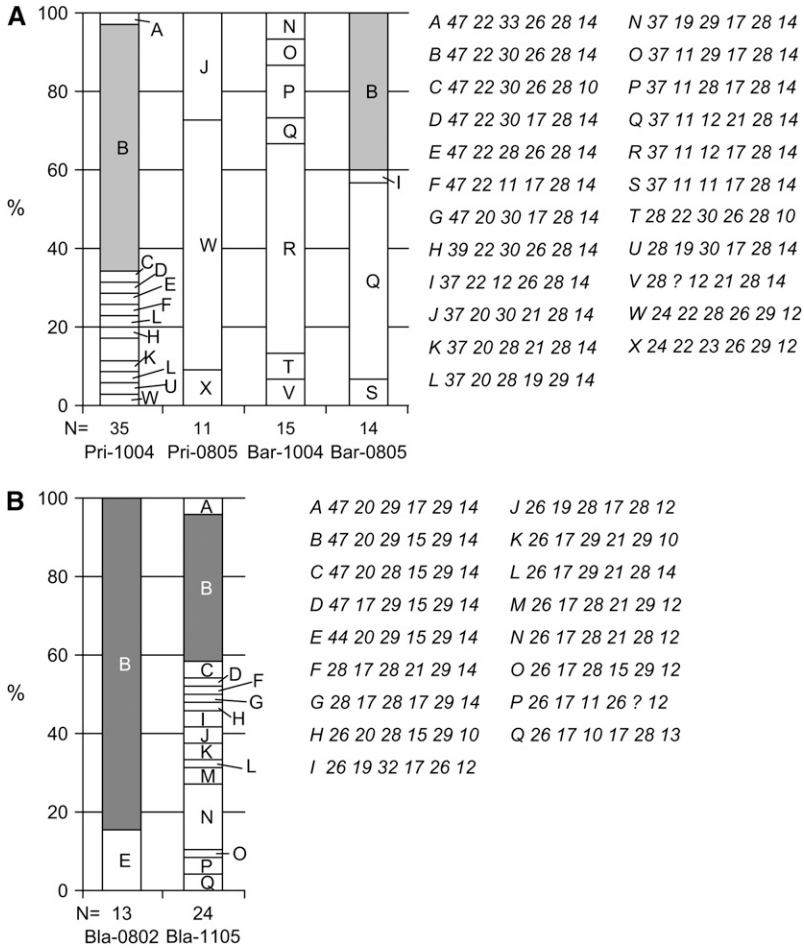

FIGURE 4.—Multilocus genotypes in the Primel/Sainte-Barbe and Le Blanc populations at two time points. (A) Haplotype frequencies in Primel (Pri) and Sainte-Barbe (Bar) in October 2004 and August 2005. *N*, number of genotyped individuals. Haplotypes are identified by their alleles (number of repeat) at each locus in the following order: *II-R*, *V-L*, *II-L*, *III-R*, *IV-L*, and *X-R*. Each haplotype is identified by a letter code common to both locations (PriBarA–X); haplotype PriBar-B (light shading) is found in both Pri-1004 and Bar-0805 samples. (B) Haplotype frequencies in Le Blanc in 2002 and 2005. Each haplotype is identified by a letter code (BlaA–Q). Haplotype Bla-B (dark shading) was found on both dates.

Selection against heterozygotes and recombinants?: Outcrossing rates could be variable over time, for example, seasonal or with a positive correlation between outcrossing and density. However, this cannot explain an increase in linkage disequilibrium. One possible alternative explanation would be partial reproductive isolation between the two haplotypes, either prezygotic (a lower rate of mating) or postzygotic (lower fitness of progeny from a cross between the two haplotypes). The former possibility was contradicted by the fact that recombinants between the two haplotypes were found, including some heterozygotes. To test the hypothesis of postzygotic isolation, we crossed two strains representing the two major haplotypes at the first time point (JU360 and JU361) and compared self- *vs.* cross-progeny for progeny number and survival in the F_2 generation. Embryonic lethality was high (4%) in the F_2 progeny of F_1 self- and cross-progeny; however, no significant difference was found between self- and cross-progeny in terms of brood size (means: 191.0 and 205.7, respectively; *P*-value: 0.42), embryonic lethality, or other obvious defects (supplemental Table S4 at http://www.genetics.org/supplemental/).

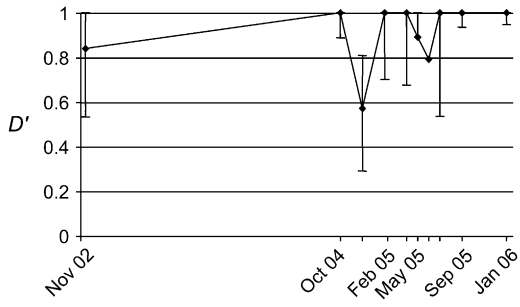

FIGURE 5.—Evolution of linkage disequilibrium over time in the Franconville population. Linkage disequilibrium *D'* was measured at different time points. Error bars delimit the 95% confidence interval.

DISCUSSION

The present results confirm previous findings on *C. elegans* genetic diversity, geographical structure, and outcrossing rate based on heterozygote frequency. Most interestingly, they provide evidence for (1) highly dynamic populations, sometimes undergoing extinction and recolonization events, and (2) maintenance of linkage disequilibrium between loci over several years. We discuss the possible consequences of this dynamic aspect of *C. elegans* populations for this worm's genetic and phenotypic evolution.

Spatio-temporal structure of *C. elegans* populations and metapopulation dynamics: The systematic sampling of several locations at different time points allowed us to obtain a dynamic picture of *C. elegans* natural populations. The metapopulation dynamics that we observed involve several temporal and spatial scales. The temporal scale of population turnover appears to be a few weeks or months, consistent with *C. elegans* being found in ephemeral habitats, such as fruits rotting below their tree or decaying snails, or, at a longer timescale, compost heaps and the surroundings of trees during the ripening period. The spatial scale of founding individual migration appears to be >1 m, with significant migration over very long distances and no correlation between genotypic divergence and geographic distance (Barrière and Félix 2005; Cutter 2006). High selfing rates may increase apparent levels of structure by reducing the effective number of migrants (Nordborg 1997).

Different migration modes may operate at different spatial scales. Nematodes in the soil are able to move by themselves over a mean distance of 15 cm, and sometimes 1 m, in a month (Robinson 2004), which could explain the lack of structure at small scale. At a larger scale, migration may occur through vectors such as invertebrate associates (snails, isopods, etc.; Kiontke and Sudhaus 2006); the sample HerD-1105, recovered from a fly bait (see Table 1), supports this idea. Wind was described to be a potential long-distance (hundreds of meters to a few kilometers) migration vector for plant parasitic nematodes (White 1953), and dust storms can be responsible for considerable movement of dauer larvae. Indeed, dauers of *C. elegans* can survive dessication for several days at room temperature (Kiontke and Sudhaus 2006) and may use migration vectors that would appear unfit at first glance. Since we found a high density of *C. elegans* in rotting fruits, fruits may also be an efficient way to migrate—with the help of humans, flies, or birds—over long distances. Overall, the association of *C. elegans* with human-related habitats indicates that human activities could be responsible for large-scale migration. The fact that *C. elegans* was seldom found in soil supports the idea of a patchy distribution of the species, with migration being a critical survival factor.

Outcrossing and maintenance of strong linkage disequilibrium: Three different measures provide information on the relative occurrence of selfing and outcrossing: heterozygote frequency, male frequency, and linkage disequilibrium. Heterozygote and male frequencies provide a measure at the short temporal scale of the previous generations. Our new estimates of heterozygote frequencies, yielding a global estimate of 1.7% outcrossing (C.I.: 1.1–2.5%), confirm our previous ones (Barrière and Félix 2005). It is improbable that many of these heterozygotes are due to mutational events, because half of them are heterozygotes at several loci (supplemental data set S1 at http://www.genetics.org/supplemental/). If we consider selfing to be constant in the species, this 1.7% outcrossing rate predicts a male frequency of 0.85% (C.I.: 0.55–1.25%), not including spontaneous males, whereas the observed male frequency is only 0.18% (95% C.I.: 0.05–0.45%). We cannot completely rule out that some males were missed in our sampling procedure, but the discrepancy suggests that outcrossing rates vary over time and between different populations, as also suggested by the variation in outcrossing rate estimates among populations (Table 2).

Much more divergent is the 100-fold lower outcrossing rate estimate (10^{-4}) based on static estimates of linkage disequilibrium between loci in a local population (Barrière and Félix 2005) or among worldwide isolates (Cutter 2006). We find a very strong linkage disequilibrium between loci located on different chromosomes for most samples (supplemental Table S3 at http://www.genetics.org/supplemental/). Cutter (2006) found similarly strong linkage on a worldwide scale (multilocus linkage disequilibrium $I_A^S = 0.29$). Within a population of constant size, significant nonrandom associations between loci can appear by mutation and drift. In rapidly growing populations, like those undergoing metapopulation dynamics, such nonrandom associations are expected to be rare (Slatkin 1994). Therefore, the observed linkage disequilibrium in *C. elegans* must have been present since the foundation of the population, possibly after colonization by two genotypes. Such a high level of linkage disequilibrium would then be expected to decay over time as a function of the outcrossing rate.

In our temporal surveys, we found no evidence of linkage disequilibrium decay over 3 years in the Franconville population. These observations are not compatible with the outcrossing rate estimated from heterozygote frequencies, unless the generation time is more than half a year. We found *C. elegans* mostly in the dauer stage, which could be responsible for a huge increase in generation time (in laboratory conditions, dauers may live up to 8 months; C. Braendle, personal communication). However, the occurrence of only six generations in 38 months is improbable.

Several mechanisms can explain the discrepancy between the short-term outcrossing rate measured by heterozygote frequency and the maintenance of high linkage disequilibrium. Population structure may explain high linkage disequilibrium among, but not within, populations. The sampled populations, however, could be sink populations, receiving a constant flow of migrants from two populations, each monomorphic for one major haplotype: a Wahlund effect (Wahlund 1928) could then explain the absence of decay of linkage disequilibrium at a given time point. Linkage should ultimately decay over time in this population, given that some sampled individuals appeared to be part of the reproductive pool (non-dauer

stages). Our observations, however, may reflect stochastic effects associated with a small effective population size. Finally, an alternative hypothesis that might explain our observations is selection acting against the cross-progeny (F_1 or later generations). In laboratory conditions, we failed to find evidence of a strong effect on brood size of recombination of the two major Franconville haplotypes, yet it is possible that another character affecting fitness in natural conditions may be affected. Outbreeding depression was indeed observed between *C. elegans* isolates, including within some of our local sets (Dolgin *et al.* 2007). Thus, a possible explanation for maintenance of linkage disequilibrium is selection against heterozygotes or recombinants.

By comparison, in *D. melanogaster*, linkage disequilibrium is very low and decays within 1 kb (Long *et al.* 1998). Even in a highly selfing species like *Arabidopsis thaliana*, linkage disequilibrium is lower than in *C. elegans*, at least on a large geographical scale. Indeed, at this global scale, linkage disequilibrium is undetectable between different chromosomes and decays within ~50–250 kb for linked loci (Nordborg *et al.* 2002, 2005); linkage disequilibrium in a short region of 170 kb is $I_A^S = 0.179$ (Haubold *et al.* 2002), weaker than in the complete genome of *C. elegans* and, unlike in *C. elegans* (Cutter 2006), some of it may be the result of spatial population structure (Schmid *et al.* 2006). At a small scale within a patch of *A. thaliana*, linkage disequilibrium appears extensive, as in *C. elegans* (Bergelson *et al.* 1998; Nordborg *et al.* 2002; Stenoien *et al.* 2005).

Possible consequences of *C. elegans* population dynamics on its phenotypic evolution: Both demographic and genetic results allow us to infer several consequences for the genetic and phenotypic evolution of *C. elegans*. The low outcrossing rate implies that alleles occur mostly in a homozygous state; hence, purging of strongly deleterious recessive mutations should occur more readily than in outcrossing populations. Populations experiencing bottlenecks are likely to fix slightly deleterious mutations by genetic drift, but the strong reexpansion regimes that follow may allow compensatory mutations to occur. Recent experimental evolution studies in *C. elegans* revealed that significant increase in fitness could already be seen after 10 generations of population reexpansion (Estes and Lynch 2003). A transient loss of fitness, or of robustness of a given phenotypic character, followed by compensatory evolution, may be a frequent mechanism of exploration of the genotype–phenotype landscape in *C. elegans*. Furthermore, the almost exclusive selfing of *C. elegans* would be expected to favor co-evolution of its entire genome, thus resulting in outbreeding depression when outcrossing actually occurs (Agrawal 2006).

In addition, if the *C. elegans* metapopulation comprises source and sink populations, adaptation in the sink populations, which are doomed to extinction, is not relevant to future generations of the species as a whole, which would adapt only to source environments. Identification of sink and source environments is thus crucial for the study of adaptive traits of *C. elegans*.

The very low overall genetic diversity of *C. elegans* (Barrière and Félix 2005; Haber *et al.* 2005; Sivasundar and Hey 2005; Cutter 2006) cannot be explained by the mere twofold reduction due to selfing; however, the observed metapopulation dynamics, in association with high rates of selfing, may result in selective sweeps that affect the whole genome and thus drastically reduce overall genetic and phenotypic diversity (Charlesworth and Charlesworth 1998). The population dynamics of *C. elegans* will thus affect molecular evolution patterns by reducing genetic diversity, increasing linkage disequilibrium, and potentially allowing the fixation of slightly deleterious mutations, which then may be compensated at the same or another locus.

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