# Population Dynamics Inferred From Temporal Variation at Microsatellite Loci in the Selfing Snail *Bulinus truncatus*

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ABSTRACT

We analyzed short-term forces acting on the genetics of subdivided populations based on a temporal survey of the microsatellite variability in the hermaphrodite freshwater snail *Bulinus truncatus*. This species inhabits temporary habitats, has a short generation time and exhibits variable rates of selfing. We studied the variability over three sampling dates in 12 Sahelian populations (1161 individuals). Classical genetic parameters (estimators of $H_o$, $H_e$, $f$, selfing rate and *Fst*) showed limited change over time whereas important temporal changes of allelic frequencies were detected for 10 of the ponds studied. These variations are not easily explained by selection, sampling drift and genetic drift alone and may be due to periodic migration. Indeed the habitats occupied by the populations studied are subject to large temporal fluctuations owing to annual cycles of drought and flood. In such ponds our results support a demographic model of population expansions and contractions under which available habitats, after the rainy season, are colonized by individuals originating from a smaller number of refuges (areas that never dry out in the deepest parts of the ponds). In contrast, selfing appeared to be an important force affecting the genetic structure in permanent ponds.

ONE of the main concerns of population genetics is the distribution of genes within and between populations of a given species. The distribution of neutral genes in subdivided populations is generally considered under a balance between genetic drift and migration (review in SLATKIN 1985). Genetic drift results in local differentiation, whereas migration may prevent divergence of populations. The relative influence of such forces can often be inferred from the geographic distribution of allelic frequencies (SLATKIN and BARTON 1989). However, other forces can modify the genetic structure expected under these ideal conditions. For example, selfing leads to a decrease (increase) of variation within (among) subpopulations when compared to outcrossing (MARUYAMA and TACHIDA 1992; CHARLESWORTH *et al.* 1993; JARNE 1995). Extinction and recolonization processes may also modify the distribution of genetic variability in subdivided populations (SLATKIN 1977; MARUYAMA and KIMURA 1980; SLATKIN 1985; WADE and MCCAULEY 1988; BARTON and WHITLOCK 1996), although this is not universal (RANNALA and HARTIGAN 1995; RANNALA 1996). Studies of these effects have been mostly theoretical to date, and we have few examples in which the details of the forces at work have been investigated (see MCCAULEY 1989; BARTON and WHITLOCK 1996).

Temporal analyses may allow insights into the dynamics of natural populations beyond those offered by geographic surveys. Such analyses not only give the opportunity to validate results obtained through geographic surveys at only one point in time but also provide information on forces responsible for genetic changes on a short time scale of a few generations. Although this point has received theoretical attention (*e.g.*, PAMILO and VARVIO-AHO 1980; WAPLES 1989a,b), most empirical studies have been concerned with detecting the influence of selection (GYLLENSTEIN 1985; MUELLER *et al.* 1985), and few have explicitly considered the possible effects of selfing, migration or extinction and recolonization processes (but see LESSIOS *et al.* 1994).

Tropical freshwater snails exhibit population dynamic patterns that make them well suited as subjects for a study of the evolutionary forces affecting the distribution of genes within and between populations over a short time scale. In the Sahelian area, they colonize natural ponds or irrigation systems, which are patchily distributed and experience annual fluctuations (BROWN 1994). With cycles of drought and flood, some ponds (temporary) contract and expand and could even sometimes disappear, whereas others (permanent) show little variation over time. The tropical snail *Bulinus truncatus* occupies the shallow margins of these habitats. Previous studies have shown that its selfing rate is high in all populations studied (see VIARD *et al.* 1997). A geographic survey of microsatellite variability in 14 populations from Niger (VIARD *et al.* 1996) showed variable genetic structures, with one permanent pond markedly subdivided into local demes, whereas semipermanent and temporary ponds showed weaker ge-

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netic structure. This suggests that selfing and migration may be the prominent forces shaping the distribution of genetic variability in the former and the latter populations, respectively.

This paper aims to further characterize the forces shaping the distribution of genes among snails in these habitats. Our analysis is based on a temporal survey covering a full cycle of dry/rainy seasons. As the generation time of *B. truncatus* is relatively short (6–8 weeks in laboratory conditions), we were able to analyze the evolution of the genetic variability across generations. Such an analysis is possible only if highly polymorphic markers are available. This is especially the case in inbreeding populations, such as those of *B. truncatus*, which are less variable than random-mating populations (CHARLESWORTH *et al.* 1993; JARNE 1995). We conducted our analysis using four highly polymorphic microsatellite markers (VIARD *et al.* 1996). We surveyed 12 populations from Niger. Each population was sampled three times over the course of a year.

## MATERIALS AND METHODS

**Species studied and sampling:** The freshwater snail *B. truncatus* (Gastropoda, Pulmonata) is distributed over most of Africa, several Mediterranean islands and parts of the Middle East (BROWN 1994). Its biology is rather well known, as it acts as an intermediate host for various species of Schistosoma, the agents of bilharziasis in Africa (BROWN 1994). The selfing rates in *B. truncatus* are high (VIARD *et al.* 1997). Freshwater environments in the tropics have limits varying in time, due to cycles of flood and drying. A given site, repeatedly sampled in time, does not have exactly the same geographic location. Snails were collected three times from each of 12 sites (Figure 1, Table 1) in Niger (sample A: February 22 to March 2, 1994; sample B: January 11 to 18, 1995; and sample C: February 20 to 24, 1995). The sampling dates correspond to the middle of the dry season, when the surface area of the temporary ponds was still large. Ten populations from 1994 were previously analyzed by VIARD *et al.* (1996; see Table 1). Kobouri (sample B) and Boyze I (sample C) were not collected, because the location could not be reached and no snails were observed, respectively. Ponds were classified into three categories according to water availability over the year as permanent (P, *i.e.*, no change in water availability over the year), semipermanent (SP, *i.e.*, with water for >6 mo, but with large fluctuations in water availability) and temporary (T, *i.e.*, with water for <6 mo) (Table 1). Several populations were collected in large ponds (more than several km$^2$) such as Boyze, Mari and Namaga or when there were artificial limits, such as a dam (*e.g.*, Tera). The samples were chosen to allow the genetic variation to be detected at various geographic scales, ranging from within ponds to between ponds at distances of several hundred of kilometers (Figure 1, Table 1). The high selfing rates in *B. truncatus* might generate some microgeographic subdivisions, because individuals sampled over a small area may be closely related. The possible confounding effects of local subdivision were avoided by sampling over an area large enough to include many families. Snails were hand-collected by three to four persons for a period of 30 min on average over a large area (500 m$^2$). Live snails were brought to the laboratory in Niamey and then transported to France stored in liquid nitrogen.

**Microsatellite analysis:** DNA extraction was performed as described in JARNE *et al.* (1992). Four microsatellite loci (BT1, BT6, BT12 and BT13) were analyzed as described in VIARD *et al.* (1996), except that BT1 and BT12 were coamplified. The only modification was to include both pairs of primers and to decrease the annealing temperature during PCR to 51°.

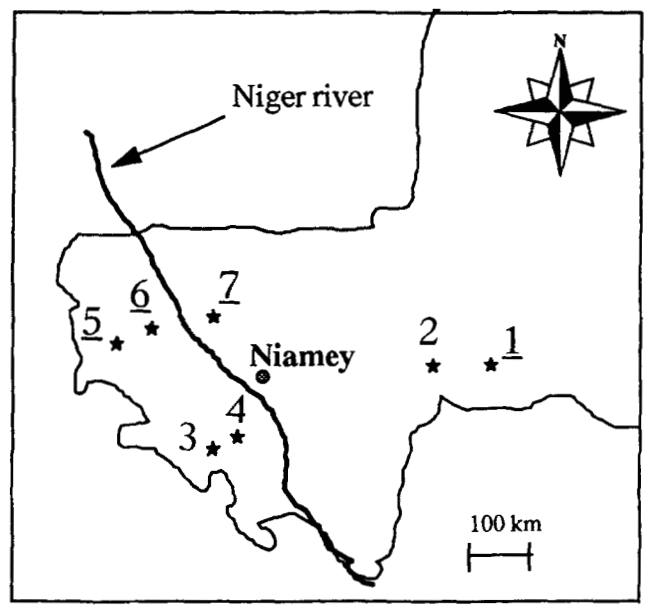


FIGURE 1.—Map of southwestern Niger with sample sites. Doubalma, Bala and Kobouri are 2, 3 and 4, respectively. The underlined numbers indicate ponds where more than one sample was collected: 1, 5, 6 and 7 are Boyze, Tera, Namaga and Mari, respectively. Boyze I and Boyze II are separated by 3 km, Mari Sud and Mari Nord are separated by 0.7 km and Tera D and Tera R are separated by 0.5 km. Namaga W is separated from Namaga B and Namaga PM by 4 and 3.5 km, respectively, and Namaga B is 1.5 km from Namaga PM.

**Statistical analysis:** For each population and sampling date, we calculated the allelic frequencies, the mean number of alleles ($n_{all}$), the observed heterozygosity ($H_o$) and the gene diversity ($H_e$). Tests for deviations from Hardy-Weinberg expectations, genotypic linkage disequilibrium for each population and sampling date and tests for differentiation among populations at each sampling date were computed using GENEPOP 2.0 (RAYMOND and ROUSSET 1995b). The estimator $\hat{f}$ of $F_{is}$ was computed according to WEIR and COCKERHAM (1984) using GENEPOP 2.0 (RAYMOND and ROUSSET 1995b). We also estimated the selfing rates using the relationship $F_{is} = S/(2 - S)$, as this relation holds regardless of the mutation model (ROUSSET 1996).

The temporal variation of allelic frequencies between each pair of samples (*e.g.*, A *vs.* B) within each population were also analyzed using a homogeneity test computed as an exact test (GENEPOP 2.0; RAYMOND and ROUSSET 1995b). The allelic frequencies were deemed to be significantly different when the probability values of the exact test were <0.05. However, both genetic and sampling drift may occur between two sampling dates within a given population, which is not accounted for by classical homogeneity tests. We therefore also used a method developed by WAPLES (1989b) to compare each pair of sampling dates for each locus, when the probability value of the exact test was <0.05. This method takes into account genetic and sampling drift effects, such that significant changes in allelic frequencies must be explained by something besides sampling or genetic drift. The method makes

**TABLE 1**

**Population characteristics and within-population polymorphism**

| Population | Type of habitat | A | | | | B | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $N$ | $n_{all}$ | $H_o$ | $H_e$ | $N$ | $n_{all}$ | $H_o$ | $H_e$ | $N$ | $n_{all}$ | $H_o$ | $H_e$ |
| Boyze I | P | 30 | 1.75 | 0.08 | 0.24 | 32 | 2.50 | 0.03 | 0.21 | 35 | 2.25 | 0.04 | 0.22 |
| Boyze II | P | 25 | 2.25 | 0.03 | 0.28 | 32 | 2.50 | 0.06 | 0.31 | - | - | - | - |
| Doubalma | T | 12 | 1.50 | 0.04 | 0.10 | 30 | 2.50 | 0.01 | 0.24 | 37 | 2.00 | 0.02 | 0.16 |
| Bala | T | 30 | 2.25 | 0.00 | 0.21 | 36 | 1.75 | 0.01 | 0.21 | 30 | 2.75 | 0.01 | 0.20 |
| Kobouri | P | 19 | 2.50 | 0.03 | 0.31 | - | - | - | - | 12 | 2.00 | 0.02 | 0.28 |
| Tera R | P | 36 | 3.50 | 0.01 | 0.40 | 30 | 6.25 | 0.02 | 0.55 | 36 | 8.00 | 0.09 | 0.64 |
| Tera D | T | 13 | 3.75 | 0.00 | 0.51 | 10 | 3.00 | 0.00 | 0.49 | 48 | 5.75 | 0.02 | 0.52 |
| Namaga PM | SP | 32 | 7.75 | 0.11 | 0.66 | 34 | 6.50 | 0.08 | 0.67 | 32 | 8.50 | 0.18 | 0.69 |
| Namaga B | SP | 24 | 6.75 | 0.16 | 0.75 | 32 | 7.75 | 0.14 | 0.68 | 53 | 8.75 | 0.08 | 0.68 |
| Namaga W | SP | 39 | 6.50 | 0.01 | 0.67 | 10 | 3.00 | 0.09 | 0.50 | 36 | 3.50 | 0.01 | 0.36 |
| Mari Sud | SP | 32 | 5.25 | 0.18 | 0.65 | 32 | 7.00 | 0.14 | 0.66 | 36 | 8.50 | 0.14 | 0.74 |
| Mari Nord | SP | 31 | 6.50 | 0.16 | 0.71 | 32 | 7.75 | 0.19 | 0.72 | 36 | 8.25 | 0.15 | 0.70 |
| Total | | 323 | | | | 310 | | | | 528 | | | |

A, B and C refer to the three sampling dates. P, permanent; SP, semipermanent; T, temporary; $N$, $n_{all}$, $H_o$ and $H_e$ are the sample size, the mean number of alleles, the observed heterozygosity and the gene diversity, respectively. -, sample not available. 1994 samples (A) of the underlined populations have been studied in VIARD *et al.* (1996).

some assumptions regarding the sampling plan, the effective size of populations and the number of generations between two samples. Analyses were conducted according to sampling plan II (individuals taken before reproduction) of WAPLES (1989b), because *B. truncatus* has a high fertility and a continuous reproduction whether the ponds were contracting or expanding. As the effective size ($N_e$) is unknown in this species, each test was performed for four different values of $N_e$ (50, 100, 1000 and 5000). We tested for such low effective sizes as 50 and 100 to take into account both the high selfing rates estimated in this species (VIARD *et al.* 1997) and the likely variation of population size over time. With a selfing rate of one, the effective size is expected to be halved (POLLAK 1987). If the effective population size varies over time and multiple samples are taken, WAPLES (1989b) suggested using a function of the harmonic mean of the effective size of each generation. An effective population size of 50 probably underestimates the actual value, even if there is a high degree of selfing, as the actual number of snails in the ponds was usually very large (see DOUMS *et al.* 1997). A significant WAPLES' test with these low values of $N_e$ therefore suggests that even strong genetic drift effects associated with fluctuating effective size and selfing cannot explain the observed variation. Overall, this made our test of the variation in allelic frequencies conservative. Based on laboratory experiments, field observations and the literature (BETTERTON *et al.* 1988; VÉRA *et al.* 1995), the number of generations between samples A and B was assumed to be four, and between samples B and C was assumed to be one. Analyses were performed using formulas derived by WAPLES (1989b). Calculations were made using both a specifically written Turbo Pascal program and Mathematica 2.2 (WOLFRAM 1991). As *B. truncatus* is a highly inbred species, we also tested for the temporal stability of multilocus genotypic distributions within populations using an exact test (GENEPOP 2.0, Struc Option; RAYMOND and ROUSSET 1995a) when temporal stability can be assumed from both the homogeneity and WAPLES' tests.

We analyzed the genetic structure among populations within, or between, ponds using the estimator $\hat{\theta}$ of $F_{st}$ (WEIR and COCKERHAM 1984), using GENEPOP 2.0 (RAYMOND and ROUSSET 1995b) and Fstat 1.2 (GOUDET 1995). Two measures of the correlation of genes between individuals within a population were calculated using samples from different times. First, we pooled the different populations from one pond and calculated $\hat{\theta}_{pond}$ between the different sampling dates (*e.g.*, $\hat{\theta}$ between samples A and B for the pond Namaga, constituted by the three populations Namaga PM, Namaga B and Namaga W pooled together). Second, we calculated $\hat{\theta}_{pop}$, which measures the differentiation between samples from two dates, for one population, within a pond (*e.g.*, $\hat{\theta}$ for Namaga PM between samples A and B). For each sampling date, we also tested for isolation by distance (SLATKIN 1993), analyzing the independence between geographic and genetic distances. Geographic distances among and within ponds were the shortest distances measured on a map and in the field, respectively. Genetic distances were $\hat{\theta}$ values. The null hypothesis of independence between geographic and genetic distances was tested by looking at the Spearman's rank correlation coefficient against the hypothesis of a positive correlation expected under isolation by distance. The observed correlation coefficient was compared to the distribution of correlation coefficients over Mantel-like permutations of the genetic and geographic distance matrices performed using GENEPOP 2.0 (RAYMOND and ROUSSET 1995b).

Our analysis often implied replicated independent tests, some of which may be significant by chance alone. We thus used Fisher's method for combining independent results (SOKAL and ROHLF 1995). A significant combined probability means that the null hypothesis is violated in at least one of the tests performed.

Microsatellite loci probably evolve under a stepwise mutation model, although an infinite alleles model cannot always be rejected (review in JARNE and LAGODA 1996). We therefore did not distinguish between the two models. This has no bearing on the variation of allelic frequencies over a short time scale and the estimates of the selfing rates. However this may influence our perception of the genetic differentiation among populations (SLATKIN 1995; ROUSSET 1996), although to a limited extend in the populations studied (VIARD *et al.* 1996).

## RESULTS

**Temporal stability of genetic structure within populations:** A large amount of polymorphism was observed

**TABLE 2**

**Fixation index and selfing rate estimates**

| | A | | B | | C | |
|---|---|---|---|---|---|---|
| | $\hat{f}$ | $S$ | $\hat{f}$ | $S$ | $\hat{f}$ | $S$ |
| Boyze I | 0.69 | 0.82 | 0.85 | 0.92 | 0.84 | 0.91 |
| Boyze II | 0.88 | 0.94 | 0.80 | 0.89 | -[a] | - |
| Doubalma | 0.60 | 0.75 | 0.97 | 0.98 | 0.88 | 0.94 |
| Bala | 1.00 | 1.00 | 0.96 | 0.98 | 0.85 | 0.92 |
| Kobouri | 0.91 | 0.95 | - | - | 0.93 | 0.96 |
| Tera R | 0.97 | 0.98 | 0.96 | 0.98 | 0.86 | 0.92 |
| Tera D | 1.00 | 1.00 | 1.00 | 1.00 | 0.97 | 0.98 |
| Namaga PM | 0.84 | 0.91 | 0.89 | 0.94 | 0.74 | 0.85 |
| Namaga B | 0.79 | 0.88 | 0.80 | 0.89 | 0.89 | 0.94 |
| Namaga W | 0.98 | 0.99 | 0.84 | 0.91 | 0.96 | 0.98 |
| Mari Sud | 0.72 | 0.84 | 0.79 | 0.88 | 0.81 | 0.90 |
| Mari Nord | 0.77 | 0.87 | 0.74 | 0.85 | 0.79 | 0.88 |

[a] Sample not available.

over the 12 populations (1161 individuals) studied, since 6, 13, 32 and 46 alleles were observed at loci BT1, BT6, BT12 and BT13, respectively. Allelic frequencies at the four loci are provided in the Appendix. The number of individuals studied, the mean number of alleles, mean heterozygosity and mean gene diversity across loci are given for each population in Table 1.

The deviations of genotype frequencies from Hardy-Weinberg expectations were highly significant for all populations, regardless of the sampling date. The associated probabilities were always $<0.03$ and 133 of 144 tests had a probability $< 0.01$. These deviations were due to heterozygote deficiencies (Table 1) as measured by $\hat{f}$ values (Table 2). The smallest observed $\hat{f}$ was 0.6 in Doubalma (sample A), with other values always exceeding 0.74. These high $\hat{f}$ values produced high estimates of the selfing rate $S$, $>0.85$ in most cases (Table 2). A test for genotypic disequilibrium between pairs of loci was possible in 216 cases. Of these, 40 tests proved significant at the 5% level (data not shown). However, disequilibria never occurred between the same pairs of loci for samples taken at different times or from different populations. No significant disequilibrium was detected in samples from Bala, in Mari Sud and Mari Nord (samples A and B) and Boyze I and Doubalma (samples B and C).

The stability of allelic frequencies over time was first examined using an exact test (Table 3). We failed to detect a significant variation in a total of 44 of 112 tests. Two populations, Boyze II and Bala, did not exhibit any difference among the three sampling dates, even at the most polymorphic loci. In these populations, an exact test was used to compare the multilocus genotypic structure among each pair of samples. This test showed that the multilocus structure did not differ with time ($P > 0.36$, SE $< 0.05$). Other populations always exhibited significant differences when taking into account all loci and samples with homogeneity tests (Table 3). The differences were also generally significant at loci BT12 and BT13 when analyzing pairs of samples, whereas they were usually not at the least polymorphic locus BT1: only four tests of 25 were significant at BT1 whereas almost 75% of the tests were significant at the other loci (Table 3). The probability value of the exact test was significant ($<0.05$) in 68 locus-population-sampling date combinations. We used the method of WAPLES (1989b) in these 68 cases for each of four $N_e$ values (see MATERIALS AND METHODS and Table 3). Although eight and five tests of 68 were not significant for $N_e = 50$ and for $N_e = 50$ or 100, respectively, WAPLES' method generally gave the same result as the exact test. In each population the differences were significant at least for one locus with WAPLES' test, whatever the value of $N_e$. A consequence is that the observed differences in allelic frequencies do not appear to be explained by genetic drift or sampling drift alone. More tests were significant between samples A and C (27 of 38) than between samples A and B (22 of 38), and also between samples B and C (19 of 36), when using exact tests. This suggests that the temporal instability of allele frequencies is positively related to the time elapsed between samples.

**Genetic variability among populations:** The probability values obtained using the exact test for differentiation and the $\hat{\theta}$ values are presented in Table 4. A large amount of differentiation was obvious over all populations for each sampling date ($P < 10^{-5}$). This remained true for the two populations from both Boyze and Tera ($P < 10^{-5}$). The three populations of Namaga showed a significant differentiation over all loci ($P < 0.01$), though they exhibited low $\hat{\theta}$, except for sample C. The differentiation is significant in Mari over all loci ($P < 0.01$) for the three samples, though no significant values were found for locus BT1 and BT6 in sample C, and BT13 in samples B and C. The corresponding $\hat{\theta}$ values are very low (Table 4). The temporal $\hat{\theta}_{pond}$ values were always lower than the spatial $\hat{\theta}$ value (Table 4). Although no tests are available for the difference between $\hat{\theta}$ values, this result may indicate that the genetic structure between two sampling dates in one pond is weaker than the genetic differentiation between the different populations of one pond at a given date. Moreover, the temporal $\hat{\theta}_{pop}$ values (not shown) were usually greater than the $\hat{\theta}_{pond}$ values (seven of nine, three of six and five of six cases for Namaga, Mari and Tera, respectively). This suggests that in these three ponds the genetic differentiation between two sampling dates is more important when analyzing a single site than at the level of the whole pond. On the other hand, $\hat{\theta}_{pop}$ was always lower than $\hat{\theta}_{pond}$ in Boyze. A significant pattern of isolation by distance was found for each sampling date. The probability values of the Mantel-like test for rejecting independence between the geographic and genetic distances in samples A, B and C were $10^{-4}$, $10^{-4}$ and $3.10^{-3}$, respectively.

**TABLE 3**

**Temporal variation of allelic frequencies**

| | Sample A/Sample B | | | | Sample A/Sample C | | | | Sample B/Sample C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BT1 | BT6 | BT12 | BT13 | BT1 | BT6 | BT12 | BT13 | BT1 | BT6 | BT12 | BT13 | All |
| Boyze I | - | - | * | *** | 0.50 | - | (***) | *** | 0.50 | - | **** | 0.64 | **** |
| Boyze II | - | - | 0.16 | 0.77 | -- | -- | -- | -- | -- | -- | -- | -- | 0.41 |
| Doubalma | - | * | 0.32 | 0.79 | - | 0.33 | - | 0.82 | - | * | * | 0.36 | ** |
| Bala | 0.49 | - | 0.66 | 0.26 | 0.20 | - | 0.05 | 0.94 | - | - | 0.15 | * | 0.088 |
| Kobouri | -- | -- | -- | -- | - | - | ** | ** | -- | -- | -- | -- | *** |
| Tera R | 1.00 | **** | **** | **** | 1.00 | **** | **** | **** | 1.00 | **** | **** | **** | **** |
| Tera D | 0.07 | ** | * | **** | (***) | *** | **** | **** | 1.00 | *** | *** | *** | **** |
| Namaga PM | 0.25 | (*) | *** | **** | 0.06 | (**) | (**) | *** | 0.28 | 0.56 | 0.21 | * | **** |
| Namaga B | 0.81 | (*) | **** | (**) | (*) | (*) | ** | *** | 0.05 | 0.24 | *** | **** | **** |
| Namaga W | 0.47 | * | 0.54 | ** | **** | (**) | (****) | **** | ** | 0.57 | 0.18 | **** | **** |
| Mari Sud | 0.70 | 0.71 | ** | **** | 0.86 | (**) | ** | ** | 0.41 | (*) | ** | **** | **** |
| Mari Nord | 0.52 | ** | ** | ** | 0.38 | (**) | (**) | 0.78 | 0.07 | 0.30 | 0.49 | * | **** |

Probability values for rejectiong the hypothesis of stability of allelic frequencies using an exact test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 10^{-5}$. The result of WAPLES' method is given when the probability of the exact test is <0.05: ( ), significant except for $N_e = 50$; ( ), significant except for $N_e = 50$ and 100; __, not significant; all other tests were significant. -, irrelevant because of monomorphism; --, irrelevant because one of the samples was not available.

## DISCUSSION

This geographic and temporal survey of the distribution of genetic variability in *B. truncatus* demonstrates the power of microsatellites as tools for studying microevolutionary changes, even in highly selfing populations where more traditional markers, such as allozymes, often lack power because of limited polymorphism (NJIOKOU *et al.* 1993; JARNE 1995). For all sampling dates, microsatellites revealed high levels of genetic variation (number of alleles, gene diversity) and the estimated selfing rates were high in all populations (large heterozygote deficiencies). These latter values are in agreement with other more direct studies (*e.g.*, progeny array analyses; VIARD *et al.* 1997). The genetic variation was strongly structured among populations at the level of the whole study, as well as within some ponds, such as Boyze or Tera (see Table 4), although others exhibited limited structure (Mari and Namaga). As the same general patterns were observed in 1994 and in both samples from 1995, we are allowed to conclude that a geographic survey at one point in time is sufficient to resolve the general features of *B. truncatus* population genetics.

**TABLE 4**

**Spatial $\hat{\theta}$ values among all populations (All) or within ponds and temporal $\hat{\theta}_{pond}$ values**

| | | Spatial | | | Temporal | | |
|---|---|---|---|---|---|---|---|
| | *d* | A | B | C | A/B | A/C | B/C |
| Boyze | 2 | 0.30 | 0.40 | - | 0.06 | - | - |
| Tera | 2 | 0.43 | 0.26 | 0.20 | 0.10 | 0.13 | 0.07 |
| Mari | 2 | 0.01 | 0.02 | 0.00 | 0.01 | 0.01 | 0.01 |
| Namaga | 3 | 0.07 | 0.05 | 0.12 | 0.01 | 0.02 | 0.00 |
| All | 12 | 0.41 | 0.41 | 0.41 | | | |

A, B and C are the three sampling dates; *d*, number of populations per pond; -, sample not available.

The temporal genetic survey conducted in this study provides instrumental information about the relationship between genetic structure and population dynamics in *B. truncatus*. Values of $H_e$ and $\hat{f}$ appeared stable over time. However, except in panmictic populations, these parameters are expected to be correlated over time, and little can be inferred from their apparent stability. On the other hand, allelic frequencies vary significantly between sampling dates in 10 of 12 populations. Four reasons are usually given to explain temporal variations in allelic frequencies: selection, sampling drift, genetic drift and migration. Selective effects have previously been shown to influence the temporal variation of genetic structure (GYLLENSTEIN 1985; MUELLER *et al.* 1985; BARKER *et al.* 1986; ALLARD 1988; SAGHAI MAROOF *et al.* 1994). However as the loci used in this study were obtained by a random screening of the 72 chromosomes of *B. truncatus*, and as only few examples of direct selective effects acting on microsatellites are known (CHARLESWORTH *et al.* 1994), it appears unlikely that the variation is due to direct selection or linkage between a neutral and a selected locus. The temporal variation of allelic frequencies might also be explained as due to sampling or genetic drift. These two effects alone are probably not sufficient, since we showed using WAPLES' procedure that the variation is larger than what is expected from sampling effects or/and genetic drift alone. We are therefore left with only one possibility of the four mentioned above, migration of individuals.

A scheme of the joint evolution of the habitat and the population could be drawn from our spatial and temporal genetic structure analyses together with ecological data. A clearer picture of the genetic functioning

of *B. truncatus* populations appears if we consider separately samples A and B (or C), which are separated by four to five generations, that is a full year (one dry and one rainy season), from samples B and C, which are separated by about one generation. In our sampling area the few available ecological studies on the population dynamics of *B. truncatus* suggest that seasonal density fluctuations are correlated with water level and rainfall. For example, a peak of population density had been observed just after flooding, followed by a population crash before complete drying out in a semipermanent pond from Niger (VERA *et al.* 1995). Moreover, once a pond has a reduced surface or is dried out, individuals are grouped in refuges (where water is available all over the year) or aestivate into the muddy bottom of the deepest parts of the pond (BETTERTON *et al.* 1988; COULIBALY and MADSEN 1990). Ecological studies also showed that colonization during the next rainy season occurs from these refuges via torrential runoff (BETTERTON 1984). From these ecological observations and our results the following scenario can therefore be proposed for the dynamics of *B. truncatus* populations in temporary or semipermanent ponds (Figure 2A). At the end of the rainy season, the water availability (in surface) is at its maximum. The density of snails reaches high values. The ponds then recede during the dry season, and individuals from any parts of the ponds mix within the deepest areas. These serve as refuges and may or may not dry out. The whole ponds are then recolonized from these refuges by few individuals at the beginning of the next rainy season. Note that a given site may be recolonized from different refuges and that individuals from a given refuge can recolonize different sites (Figure 2A). Our sampling A and B took place when the ponds were about at their largest surface. The scenario proposed above is based on the genetic data obtained at these two dates in ponds for which more than a sample is available (*e.g.,* Mari or Namaga) as follows. Migration of individuals from refuges at the end of the rainy season is suggested by the strong variation of allelic frequencies within each population. That there is more than a single refuge (in which case we should not observe other effects than those of genetic and sampling drift) is an assumption of our scenario. This is suggested by the fact that the genetic differentiation in a whole pond at a given date is more important than the differentiation between two sampling dates. From an even more rigourous perspective, our scenario implies that important changes in allelic frequencies occur in each local population while those of the overall population (pond) remain unchanged. This could not be tested here, since the observed variability within a given pond was not representative of the whole pond (*i.e.,* the actual shoreline of a given pond is much longer than the distance over which the samples were taken). In other words, we did not sample the variability of the whole pond. A further point is that the expansion and

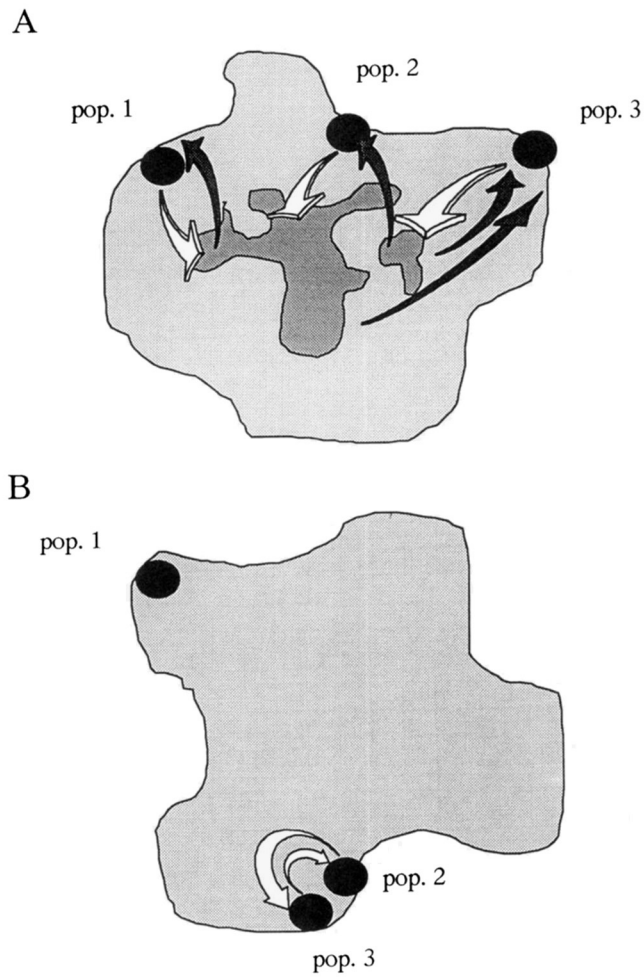


FIGURE 2.—Schematic representation of the joint evolution of populations and their habitat (see text for the genetic interpretation). (A) In temporary ponds. The areas sampled at dates A and B are indicated by the filled circles. While the ponds recede, individuals migrate toward the deepest parts of the ponds (refuges, white arrows). Several refuges may persist. Recolonization after the rainy season of each area sampled occurs from the refuges and may involve individuals from a single or from several refuges (black arrows). (B) In permanent ponds. The pond remains almost identical to itself over time. Populations exchange migrants over very short distances only (arrows).

contraction phases of the ponds should correspond to strong variations in the effective size of the populations or, in other words, to genetic bottlenecks. This certainly has some consequences on the amount of variability maintained (CHAKRABORTY and NEI 1977; CORNUET and LUIKART 1996; but see RANNALA 1996).

Samples B and C were not collected at exactly the same site, since the ponds studied receded between the two dates. Temporal effects may therefore be confounded with geographic effects at a small scale, especially in ponds with a strong genetic structure, such as Boyze or Tera. In ponds with limited genetic structure (*e.g.,* Mari), the situation can be explained as previously between samples A and B (or C). Indeed, the popula-

tions from the ponds we studied are subdivided into distinct patches that may interact via an important passive gene flow (through water flow, human activities, birds, cattle movements etc.) when water is at the highest level.

For two populations (Boyze II and Bala) we did not observe significant temporal variations of allelic frequencies. Boyze is a very deep, large pond which exhibited very limited shape and water variation over the three sampling points in time. The genetic structure created through selfing is likely to be maintained since dispersion following runoff during the rainy season is limited (Figure 2B). This is consistent with the stable pattern of the multilocus genotypic frequencies. Bala is a temporary pond and variation in allelic frequencies were expected. However the multilocus genotypic structure remained unchanged. This may be explained by the same inbred lines having been sampled over time. As the selfing rate is very high and density extremely low (data not shown), the genetic variability may be here reduced to few selfing families with limited migration.

Selfing is the major force shaping the genetic structure of populations in *B. truncatus* in permanent ponds, while both selfing and migration have to be considered in transient ponds. More specifically, the demographic scenario described above fits reasonably well the migrant pool model of recolonization in a subdivided population (SLATKIN 1985; BARTON and WHITLOCK 1996). At a larger scale, populations were markedly differentiated and exhibited a significant pattern of isolation by distance as VIARD *et al.* (1996) observed. Some seasons may indeed be dry enough that the maximum aestivation time of individuals is too short to avoid the extinction of the populations in a given pond. Recolonization could then occur from nearby ponds (see above) with colonists arising from a limited number of source ponds. This would better fit a propagule pool model of recolonization (SLATKIN 1977).

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## APPENDIX

**Allelic frequencies per locus, population and sampling date**

| Population | N | Allelic frequencies |
|---|---|---|
| Boyze I A | 60 | 1 (184)/1 (163)/0.567 (278) 0.400 (282) 0.033 (286)/0.700 (424) 0.300 (428) |
| Boyze I B | 64 | 1 (184)/1 (163)/0.063 (270) 0.031 (274) 0.656 (278) 0.234 (282) 0.016 (286)/0.090 (420) 0.820 (424) 0.090 (428) |
| Boyze I C | 70 | 0.029 (183) 0.971 (184)/1 (163)/0.271 (278) 0.543 (282) 0.186 (286)/0.060 (420) 0.870 (424) 0.070 (428) |
| Boyze II A | 50 | 1 (184)/1 (163)/0.042 (278) 0.708 (282) 0.250 (286)/0.110 (424) 0.410 (428) 0.390 (432) 0.090 (436) |
| Boyze II B | 64 | 1 (184)/1 (163)/0.078 (278) 0.516 (282) 0.391 (286) 0.015 (290)/0.060 (424) 0.380 (428) 0.470 (432) 0.090 (436) |
| Doubalma A | 24 | 1 (184)/1 (165)/1 (262)/0.040 (284) 0.750 (296) 0.210 (424) |
| Doubalma B | 60 | 1 (184)/0.207 (143) 0.793 (165)/0.933 (262) 0.067 (266)/0.032 (284) 0.068 (292) 0.690 (296) 0.020 (408) 0.190 (424) |
| Doubalma C | 74 | 1 (184)/0.068 (143) 0.932 (165)/1 (262)/0.040 (284) 0.027 (292) 0.635 (296) 0.300 (424) |
| Bala A | 60 | 0.967 (184) 0.033 (186)/1 (116)/0.767 (258) 0.233 (262)/0.033 (276) 0.733 (280) 0.200 (284) 0.034 (288) |
| Bala B | 72 | 1 (184)/1 (116)/0.810 (258) 0.190 (262)/0.633 (280) 0.300 (284) 0.067 (288) |
| Bala C | 60 | 1 (184)/1 (116)/0.042 (250) 0.028 (254) 0.792 (258) 0.111 (262) 0.027 (266)/0.056 (276) 0.736 (280) 0.181 (284) 0.027 (288) |
| Kobouri A | 38 | 1 (184)/1 (155)/0.059 (278) 0.118 (282) 0.706 (286) 0.117 (290)/0.235 (328) 0.294 (332) 0.353 (336) 0.118 (340) |
| Koubouri C | 24 | 1 (184)/1 (155)/0.417 (278) 0.583 (286)/0.250 (332) 0.583 (336) 0.042 (340) 0.125 (344) |
| Tera R A | 72 | 0.028 (180) 0.972 (184)/0.250 (143) 0.028 (149) 0.722 (161)/0.083 (238) 0.528 (290) 0.319 (294) 0.070 (298)/0.056 (344) 0.194 (376) 0.056 (380) 0.682 (384) 0.013 (388) |
| Tera R B | 60 | 0.028 (180) 0.972 (184)/0.029 (116) 0.059 (143) 0.088 (149) 0.206 (157) 0.618 (161)/0.264 (238) 0.028 (242) 0.028 (266) 0.028 (278) 0.125 (290) 0.278 (294) 0.056 (298) 0.111 (342) 0.055 (346) 0.027 (354)/0.029 (284) 0.029 (348) 0.171 (352) 0.114 (356) 0.086 (372) 0.071 (376) 0.471 (380) 0.029 (388) |
| Tera R C | 72 | 0.033 (180) 0.967 (184)/0.069 (116) 0.224 (143) 0.103 (149) 0.156 (157) 0.448 (161)/0.034 (234) 0.121 (238) 0.069 (242) 0.069 (274) 0.069 (290) 0.310 (294) 0.069 (298) 0.017 (342) 0.086 (346) 0.052 (350) 0.034 (354) 0.070 (358)/0.037 (340) 0.074 (348) 0.037 (352) 0.222 (356) 0.019 (360) 0.093 (364) 0.093 (368) 0.130 (372) 0.019 (376) 0.111 (380) 0.111 (384) 0.018 (388) 0.036 (396) |
| Tera D A | 26 | 0.154 (176) 0.077 (180) 0.769 (184)/0.077 (143) 0.692 (149) 0.231 (157)/0.077 (238) 0.077 (250) 0.154 (254) 0.538 (274) 0.077 (290) 0.077 (318)/0.078 (276) 0.692 (368) 0.230 (408) |
| Tera D B | 20 | 1 (184)/0.100 (141) 0.400 (143) 0.500 (149)/0.100 (238) 0.500 (274) 0.300 (318) 0.100 (322)/0.300 (272) 0.100 (352) 0.500 (364) 0.100 (368) |
| Tera D C | 96 | 0.021 (180) 0.979 (184)/0.106 (143) 0.681 (149) 0.191 (151) 0.022 (157)/0.021 (254) 0.146 (258) 0.188 (262) 0.021 (266) 0.198 (274) 0.385 (318) 0.021 (322) 0.010 (350) 0.010 (354)/0.042 (264) 0.344 (268) 0.021 (348) 0.188 (352) 0.063 (360) 0.260 (364) 0.062 (368) 0.020 (376) |
| Namaga PM A | 64 | 0.097 (176) 0.758 (180) 0.145 (184)/0.468 (143) 0.016 (155) 0.516 (157)/0.150 (238) 0.033 (242) 0.050 (246) 0.117 (250) 0.050 (254) 0.050 (262) 0.033 (266) 0.333 (270) 0.033 (274) 0.133 (278) 0.018 (282)/0.035 (254) 0.017 (260) 0.035 (264) 0.178 (284) 0.017 (288) 0.035 (296) 0.035 (352) 0.070 (356) 0.035 (392) 0.035 (396) 0.040 (412) 0.250 (416) 0.178 (420) 0.040 (424) |
| Namaga PM B | 68 | 0.203 (176) 0.641 (180) 0.156 (184)/0.672 (143) 0.328 (157)/0.063 (238) 0.094 (254) 0.094 (262) 0.328 (270) 0.219 (274) 0.140 (278) 0.062 (282)/0.016 (250) 0.031 (256) 0.063 (260) 0.031 (284) 0.063 (288) 0.063 (292) 0.063 (356) 0.078 (360) 0.031 (376) 0.031 (380) 0.080 (404) 0.220 (412) 0.170 (416) 0.060 (420) |
| Namaga PM C | 64 | 0.167 (176) 0.560 (180) 0.273 (184)/0.734 (143) 0.266 (157)/0.045 (238) 0.061 (250) 0.045 (254) 0.015 (258) 0.106 (262) 0.030 (266) 0.318 (270) 0.182 (274) 0.061 (278) 0.137 (282)/0.029 (256) 0.151 (260) 0.029 (264) 0.029 (284) 0.014 (288) 0.060 (292) 0.014 (352) 0.106 (356) 0.060 (364) 0.014 (368) 0.029 (376) 0.044 (380) 0.014 (396) 0.019 (400) 0.019 (408) 0.110 (412) 0.140 (416) 0.090 (420) 0.029 (424) |
| Namaga B A | 48 | 0.313 (176) 0.541 (180) 0.146 (184)/0.500 (143) 0.104 (155) 0.396 (157)/0.125 (238) 0.250 (250) 0.104 (254) 0.167 (262) 0.208 (270) 0.083 (274) 0.063 (278)/0.109 (256) 0.043 (260) 0.043 (264) 0.043 (284) 0.152 (288) 0.087 (352) 0.043 (356) 0.087 (380) 0.043 (392) 0.044 (396) 0.020 (404) 0.200 (416) 0.070 (420) 0.020 (424) |

## APPENDIX

### Continued

| Population | N | Allelic frequencies |
|---|---|---|
| Namaga B B | 64 | 0.266 (176) 0.546 (180) 0.188 (184)/0.719 (143) 0.063 (155) 0.218 (157)/0.031 (238) 0.016 (246) 0.109 (254) 0.125 (266) 0.281 (270) 0.250 (274) 0.156 (278) 0.032 (282)/0.030 (252) 0.094 (260) 0.094 (264) 0.030 (288) 0.015 (292) 0.015 (348) 0.045 (352) 0.015 (356) 0.030 (380) 0.062 (384) 0.031 (388) 0.030 (396) 0.050 (404) 0.360 (416) 0.050 (420) 0.030 (424) 0.020 (428) |
| Namaga B C | 106 | 0.110 (176) 0.020 (179) 0.660 (180) 0.210 (184)/0.690 (143) 0.020 (155) 0.290 (157)/0.020 (234) 0.090 (238) 0.020 (242) 0.080 (246) 0.070 (250) 0.110 (254) 0.050 (262) 0.170 (270) 0.170 (274) 0.200 (278) 0.020 (282)/0.020 (256) 0.100 (260) 0.010 (280) 0.050 (284) 0.070 (288) 0.030 (352) 0.110 (356) 0.040 (360) 0.010 (364) 0.010 (368) 0.080 (380) 0.020 (384) 0.030 (388) 0.040 (404) 0.060 (408) 0.090 (412) 0.230 (416) |
| Namaga W A | 78 | 0.449 (176) 0.449 (180) 0.102 (184)/0.763 (143) 0.237 (157)/0.053 (226) 0.053 (246) 0.132 (254) 0.237 (258) 0.066 (262) 0.026 (266) 0.158 (270) 0.250 (274) 0.025 (278)/0.025 (254) 0.025 (258) 0.025 (260) 0.230 (280) 0.179 (284) 0.051 (288) 0.077 (292) 0.077 (376) 0.051 (384) 0.051 (392) 0.179 (396) 0.030 (408) |
| Namaga W B | 20 | 0.600 (176) 0.300 (180) 0.100 (184)/1 (143)/0.214 (254) 0.286 (258) 0.500 (274)/0.100 (256) 0.400 (284) 0.200 (288) 0.100 (392) 0.200 (396) |
| Namaga W C | 72 | 0.917 (176) 0.083 (180)/0.944 (143) 0.056 (157)/0.028 (238) 0.306 (258) 0.083 (270) 0.583 (274)/0.390 (284) 0.030 (356) 0.500 (396) 0.040 (400) 0.030 (408) 0.010 (428) |
| Mari Sud A | 64 | 0.034 (179) 0.224 (180) 0.742 (184)/0.483 (116) 0.052 (151) 0.465 (157)/0.310 (250) 0.207 (254) 0.017 (274) 0.052 (278) 0.190 (310) 0.155 (314) 0.069 (318)/0.125 (256) 0.339 (260) 0.018 (280) 0.036 (284) 0.196 (288) 0.143 (296) 0.054 (300) 0.089 (368) |
| Mari Sud B | 64 | 0.234 (180) 0.766 (184)/0.516 (116) 0.234 (151) 0.250 (157)/0.031 (242) 0.031 (246) 0.359 (250) 0.109 (254) 0.031 (258) 0.047 (274) 0.110 (306) 0.141 (310) 0.110 (314) 0.031 (330)/0.031 (256) 0.156 (260) 0.016 (264) 0.031 (284) 0.359 (288) 0.016 (292) 0.094 (296) 0.016 (300) 0.109 (364) 0.078 (368) 0.031 (372) 0.031 (376) 0.032 (380) |
| Mari Sud C | 72 | 0.056 (179) 0.319 (180) 0.625 (184)/0.386 (116) 0.257 (151) 0.029 (153) 0.328 (157)/0.042 (238) 0.028 (242) 0.028 (246) 0.222 (250) 0.097 (254) 0.028 (258) 0.028 (262) 0014 (266) 0.083 (274) 0.028 (286) 0.042 (302) 0.056 (306) 0.181 (310) 0.090 (314) 0.013 (330) 0.013 (350)/0.097 (256) 0.264 (260) 0.028 (268) 0.056 (280) 0.042 (284) 0.208 (288) 0.028 (292) 0.083 (296) 0.056 (300) 0.028 (364) 0.110 (368) |
| Mari Nord A | 62 | 0.350 (180) 0.650 (184)/0.274 (116) 0.210 (151) 0.500 (157) 0.0016 (159)/0.016 (246) 0.242 (250) 0.129 (254) 0.048 (274) 0.145 (306) 0.129 (310) 0.145 (314) 0.146 (318)/0.050 (256) 0.267 (260) 0.150 (284) 0.100 (288) 0.033 (292) 0.083 (296) 0.017 (300) 0.050 (304) 0.033 (348) 0.151 (368) 0.033 (376) 0.033 (384) |
| Mari Nord B | 64 | 0.016 (179) 0.297 (180) 0.687 (184)/0.344 (116) 0.172 (151) 0.016 (153) 0.468 (157)/0.234 (250) 0.172 (254) 0.047 (258) 0.031 (266) 0.125 (274) 0.031 (286) 0.031 (302) 0.125 (306) 0.078 (310) 0.063 (314) 0.032 (322) 0.031 (326)/0.094 (252) 0.141 (256) 0.047 (260) 0.078 (264) 0.031 (268) 0.109 (288) 0.016 (292) 0.063 (296) 0.188 (300) 0.031 (308) 0.109 (364) 0.093 (368) |
| Mari Nord C | 72 | 0.375 (180) 0.625 (184)/0.486 (116) 0.264 (151) 0.028 (153) 0.222 (157)/0.014 (214) 0.069 (238) 0.056 (246) 0.250 (250) 0.236 (254) 0.042 (258) 0.028 (302) 0.069 (306) 0.139 (310) 0.042 (314) 0.055 (318)/0.042 (256) 0.463 (260) 0.028 (264) 0.056 (280) 0.028 (284) 0.069 (288) 0.056 (292) 0.083 (296) 0.056 (300) 0.028 (304) 0.014 (356) 0.028 (364) 0.027 (368) 0.055 (372) 0.014 (380) 0.013 (384) |

Alleles are designated in parentheses by their size in base pairs. The allelic frequencies are given for locus BT1, BT6, BT12 and BT13 separated by /. A, B and C after the population name refer to the three sampling dates. N is the number of alleles analyzed.