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SHORT COMMUNICATION

Absence of evidence for isolation by distance in an expanding cane toad (*Bufo marinus*) population: an individual-based analysis of microsatellite genotypes

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Abstract

The cane toad (*Bufo marinus*) was introduced in 1935 in Australia, where it spread rapidly. We have tested for isolation by distance by analysing at a local geographical scale a continuous population using seven microsatellite markers and an individual-based method. The matrix of pairwise individual differentiation was not significantly correlated with that of geographical distance. Regression analyses gave a low positive slope of 0.00072 (all individuals) or a negative slope of 0.0017 (individuals with a distance higher than the previously estimated mean dispersal distance). The absence of evidence for isolation by distance favours the hypothesis that the substantial differentiation and autocorrelation previously observed at enzyme loci, mainly results from discontinuities in the colonization process with founder effects occurring at the time of the establishment of new populations.

Keywords: individual based analysis, invading species, isolation by distance, local scale differentiation, microsatellite DNA, neighbourhood size

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Introduction

Understanding the evolutionary dynamic of invasive species may help to construct predictive models for future spread and design measures of biological control. The cane toad (or giant toad *Bufo marinus*) is by far the most widely and successfully introduced amphibian species (Sabath *et al*. 1981). This species is native to the American tropics and was deliberately introduced in 1935 as a biocontrol agent in Australia, where it spread across more than one million km^2 and continues to colonize new areas. This strong invading potential translates into high colonization rates ranging from one to 30 km per year (Van Beurden & Grigg 1980; Sabath *et al*. 1981; Easteal & Floyd 1986). The rapid expansion of *B. marinus* suggests that the species is very mobile, and as a result, there could be a large amount of gene flow between its populations, reducing the rate at which genetic differentiation can occur between them. Paradoxically, substantial genetic differentiation was found among Australian cane toad populations sampled sometimes over relatively short distances (e.g. $\approx 50 \times 80$ km for the Moreton bay region, Australia) (Easteal 1985). Moreover, spatial analysis of populations differentiation revealed significant autocorrelation over various distance classes at most enzyme loci (Easteal *et al*. 1985).

It remains unclear whether the geographical pattern of variation observed at enzyme loci has been shaped predominantly by an isolation by distance process due to limited dispersal, or by complex demographic events which occurred during the recent range expansion of the species in Australia (e.g. discontinuities in the colonization dynamics with founder events occurring at the time of the establishment of new populations), and to what degree this pattern has been influenced by natural selection at some enzyme loci (Easteal 1985, 1988; Guinand & Easteal 1996). Beside the possible problem of selection, the main difficulties in interpreting the previous enzyme data sets are the mixture of geographical scales and the

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different establishment times for populations. This confuses the effect of recent demographic history and isolation by distance. A clear reference to a specific model of population structure (e.g. island or isolation by distance models) is also often missing.

In this paper, we have specifically tested the occurrence of isolation by distance by analysing at a local geographical scale a continuous population of *B. marinus* established for a relatively long time. Such analysis was achieved using microsatellite markers and an individual-based method which formally refers to isolation by distance models (Rousset 1997, 2000a).

Materials and methods

Statistical treatments

In a continuous population, genetic differentiation among individuals is expected to increase with their geographical distance measured at a local scale when isolation by distance occurs (Wright 1943). This can be quantified by a generalization of the theory of *F*-statistics. Here we consider the statistic a_r, a generalization of $F_{ST}/(1 - F_{ST})$ between pairs of individuals (Rousset 2000a). When the continuous population is represented by a two dimensional lattice (i.e. fixed individual positions and no spacial density heterogeneity) and when applied on a small geographical scale, a_r is approximately linearly related to the logarithm of distance, $a_r \approx (\ln(d)/4\pi D\sigma^2) + \text{constant}$, where d is the geographical distance between two individuals, D is the density of effective individuals, σ^2 is the second moment of the dispersal distance (i.e. the mean squared parent–offspring distance), and the constant is the value of the linear approximation at d = 1 length unit. Thus, the inverse of the regression slope provides an estimate of the 'neighbourhood size' $S = 4\pi D\sigma^2$. The statistic $\hat{a}$, a multilocus estimate of a_r computed for each pair of individuals, was regressed against the logarithm of the geographical distance between these individuals, as described in Rousset (2000a) for a two dimensional model. Note that the individual-based method used here is conceptually similar to the 'subpopulation'-based isolation by distance method described in Rousset (1997). Ninety-five per cent confident intervals around the regression slope value were computed using the ABC bootstrap procedure described in DiCiccio & Efron (1996), using code written in *Mathematica* (Wolfram 1999) after the S procedures available at http://www.stat.cmu.edu. All other analyses were performed using the version 3.2 of the package GENEPOP (Raymond & Rousset 1995).

The above method presents four interesting features: (i) It avoids the arbitrary setting of geographical limits for the sampling of subpopulations, a feature particularly useful when populations are mostly continuous as is the case for the cane toad; (ii) the variation of *F*-statistics with geographical distance gives more easily interpretable information than a *F*-statistic value computed over all units; (iii) the demographic model on which this method is based makes only weak assumptions on the distribution of dispersal distances and is robust for distribution of dispersal more leptokurtic than normal, a feature commonly observed in natural populations (Rousset 1997; 2000a); and (iv) studies at a local geographical scale are more likely to yield valuable estimates because heterogeneity of demographic parameters (e.g. spatial and historical variation in the dispersal or density of individuals) are reduced, as are their influences on heterogeneity of genetic parameters such as the ones considered here (Slatkin 1993; Rousset 2000b).

Sampling and microsatellite analysis

The *Bufo marinus* continuous population studied here is located in the region of Byron Bay (28°39′00″ S 153°37′00″ E, Australia), an area located 150 km south from the populations of the Moreton Bay region studied by Easteal (1985). Cane toads were introduced near Byron Bay in 1964. The generation time being approximately equal to one year (Easteal & Floyd 1986), this corresponds to a relatively long period of establishment (≈25–35 generations). The colonization front is currently (February 1999) at Woodburn, 90 km south from Byron Bay (A. Estoup, personal observation).

For application of the above individual based regression method, it is preferable to select individuals separated by distances shorter than $\approx 20\,\sigma$, with σ^2 the average squared axial parent–offspring distance (Rousset 1997; 2000a). Assuming that the rate of growth of newly formed populations (α) was large enough to consider that the rate of colonization ρ is equal to 2σ (Easteal & Floyd 1986), σ-values can be estimated from ρ values by using the relationship $\rho = \sqrt{2\alpha}$ (Skellam 1951). Mean rate of colonization in the Byron Bay region was estimated to be 1.07 km/year (Van Beurden & Grigg 1980) and 2.5 km/year (Easteal & Floyd 1986), which translate into parental dispersal rates of 0.535 and 1.25 km/generation, respectively. A total of 120 toads (90 matures and 30 immatures with snout-urostyle length > and <90 mm, respectively) were randomly collected in February 1999 along a 20-km transect. For each capture geographical coordinates were recorded and a toe was clipped and stored in 95% ethanol. DNA extractions were performed using a Chelex-based protocol (Estoup *et al*. 1996). Seven microsatellite loci (BM101, BM121, BM229, BM235, BM239, BM279 and BM322) were genotyped using fluorescent polymerase chain reaction (PCR) and an ABI sequencing machine (Applied Biosystem, Perkin Elmer) as described in Tikel *et al*. (2000). BM101 is an unpublished locus with 5′–3′ primer sequences GTTTCAGTAGGCAGGTGAAGA and ACCCATCCTCACAAAGGTC, allelic size range between

170 and 180 bp and PCR conditions similar to those of the locus BM128 (see Tikel *et al*. 2000).

Results and Discussion

Low level of isolation by distance

The matrix of pairwise multilocus a_r values estimated for all pairs among the 120 individuals was not significantly correlated with that of geographical distance (Mantel's test, $P = 0.82$). The regression method gave a low positive slope of 0.00072, which translates into an estimate of 'neighbourhood size' S of 1389 individuals. The ABC bootstrapping procedure gave a large 95% confidence interval with 0.025 and 0.975 threshold values being S = 90 and infinity. As the linear relationship is expected to be poor for individuals separated by a very small distance (Rousset 1997), it should be appropriate to remove individuals separated by a distance lower than σ in our analysis. A low negative slope (–0.0017) was obtained when pairs of individuals closer than 0.5 km were removed. The 0.025 and 0.975 threshold values of the 95% confidence interval were S = 67 and infinity. Thus, the estimates of S themselves give no evidence for isolation by distance. A plot of pairwise genetic differentiation between individuals against logarithm of distance as well as the regression lines including or excluding individuals distant by more than 500 m are presented in the Fig. 1.

This paper describes an application with microsatellite markers of Rousset (2000a) regression method based on individual genotypes. The variance of classical F_{ST} estimators (i.e. F_{ST} computed with subpopulation as unit) decreases with increasing genetic diversity of markers (Goudet *et al*. 1996). The same trend is expected for F_{ST}-like statistic between pairs of individuals. Hence, for a given number of loci, the regression method is expected to be more accurate with microsatellites than with less variable markers such as enzymes. However, in the Byron bay region, the level of variability at microsatellite loci was only slightly higher (mean allele number and gene diversity of 2.9 and 0.51, respectively) than that at enzymatic markers in the same area (e.g. 2.1 and 0.32, Easteal 1985). This is likely to be due to a series of bottlenecks which occurred during the introduction of *Bufo marinus* in the Caribbean and Pacific Islands (Easteal 1981), and possibly during the colonization of Australia (Slade & Moritz 1998). Therefore, the potential gain of precision from using microsatellites instead of allozymes may be small in the present study.

As a matter of fact, though 120 individuals and seven microsatellite loci were analysed, the level of precision of the regression method appears to be low as suggested by the large confidence intervals. A total of 12 microsatellite loci have been optimized on cane toad (Tikel *et al*. 2000), but three of them were monomorphic in the Byron bay region and two of them were discarded from analysis due to sex linkage (results not shown). More precision would be expected by genotyping more loci and, to a lesser extent, more individuals (Rousset 2000a), but it is likely that this would not alter the general conclusion of low isolation by distance in continuous populations of *B. marinus*.

Comparison with ecological, historical and demographic data

In contrast to the high level of philopatry observed in most amphibian species (reviewed in Waldman &

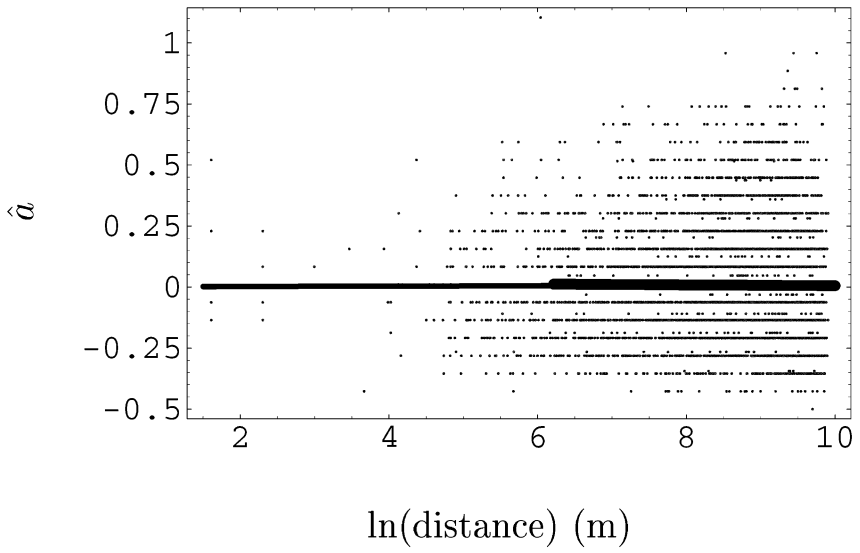

Fig. 1 Genetic differentiation in Cane toads. Pairwise genetic differentiation between individuals are plotted against logarithm of distance (in metres). All estimates for pairs of individuals at nonzero distance are shown, as well as the regression line computed from these estimates (thin line) and from estimates beyond 500 m (thick line).

McKinnon 1993), both mark-recapture and radiotracking studies have shown that cane toads rapidly move away from the location where they were captured (with individual distance per night ranging from 0 m to 1.3 km) and very seldom return (Bayliss 1994; Alford *et al*. 1995; L. Schwartzkopf & R. Alford, personal com.). This behavioural feature, as well as the high capability for rapid colonization of large areas in Australia (Easteal *et al*. 1985), are congruent with the absence of evidence for isolation by distance in the present study (Slatkin 1993).

Ecological, historical and demographic data available on *B. marinus* populations allows a rough estimation of population densities and dispersal rates, and hence that of 'neighbourhood sizes' ($S = 4\pi D\sigma^2$). Cane toad population density is known to vary in relation to time since colonization as well as to environmental features (Easteal & Floyd 1986; Freeland 1986). The population studied here is a relatively long established population located in semi-urban and agricultural areas. For this type of population, mark-recapture methods gave a density of 1500–3000 toads/km^2 in tropical and subtropical populations (Pearse 1979; Easteal & Floyd 1986) with ≈40% of mature individuals (Freeland 1986). *B. marinus* is a highly fecund species (7500–20 000 eggs/female, Alford *et al*. 1995), so it is likely that the reproductive variance is sufficiently large to substantially reduce the proportion of 'effective individuals'. However, no data are available allowing estimation of the female and male reproductive variance in cane toads. Hence, only a correction based on the proportion of mature individuals could be made on density estimates, so that mean dispersal rates of 0.535 km/year and 1.25 km/year (cf materials and methods) correspond to S-values between 2160 and 23 560 mature individuals in the Byron Bay region. Although those estimations are inaccurate and may be lower due to the reproductive variance, such large S-values are in agreement with the absence of evidence for isolation by distance (Fig. 1), and it is to be expected that the confidence intervals computed from microsatellite data would also contain large values.

Implications for the interpretation of genetic structure at different geographical and temporal scales

The absence of evidence for isolation by distance in a continuous population of *B. marinus* favours the hypothesis that the substantial genetic differentiation previously observed among populations of the Moreton Bay region, as well as the finding of significant autocorrelation over various distance classes at most enzyme loci (Easteal 1985), mainly results from discontinuities in the colonization process, with founder effects occurring at the time of the establishment of new populations. Theoretical studies have shown that founding events are likely to increase the divergence among populations (Slatkin 1977; Wade & McCauley 1988). A large increase of differentiation is particularly expected if the new populations are not immediately connected by gene flow to the main range of the species and if the population sampling includes a large proportion of recently founded populations (Slatkin 1993). Many of the populations studied in the Moreton bay region had been in existence for less than 10 years, and some for less than five, when they were sampled (Easteal & Floyd 1986). In contrast, the continuous population studied here has been established for 25–35 years and should be less affected by past demographic fluctuation than would populations closer to the colonization front. Finally, the extent and persistence of genetic differentiation may be locally enhanced by environmental features that are known to limit dispersal in *B. marinus*, especially large rivers, high density forest and mountainous areas (Zug & Zug 1979; Easteal 1985; Easteal & Floyd 1986). Studies at a local geographical scale, as in the present paper, are less prone to such environmental heterogeneity (Rousset 2000b) and, therefore, provide a clearer perspective on intrinsic limits to dispersal. We are currently completing additional population studies using microsatellite markers in order to characterize the colonization process of this invasive amphibian species in Australia.

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