

NEWS AND VIEWS

PERSPECTIVE

The coup de grâce for the nested clade phylogeographic analysis?

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Nested clade phylogeographic analysis (NCPA) has become a popular method for reconstructing the history of populations across species ranges. Ever since its invention in 1995, criticisms have been formulated, but the method, which has been regularly updated, continues to attract investigators. *Molecular Ecology* has published a large fraction of the literature on the topic — both pro and con. A recent study by Panchal and Beaumont (2007) finally allows a precise evaluation of the method by developing software that automates the somewhat complicated NCPA procedure. Using simulations of random-mating populations, Panchal and Beaumont find a high frequency of false-positives with their automated NCPA procedure (over 75%). These findings, which echo and amplify earlier warnings, appear serious enough to suggest to researchers to await further evaluation of the method. Although no other all-encompassing method such as the NCPA currently exists to evaluate phylogeographic data sets, researchers have many alternative methods to test ever more refined hypotheses.

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As pointed out by Hey & Machado (2003) and Wakeley (2003), the study of structured populations is a difficult and divided science. Two different schools of thought are recognized, the phylogeographic school, which relies on graphical representation of intraspecific phylogenetic trees or networks (e.g. Avise 2000), and the mathematical one, which avoids this step and relies instead on more abstract mathematical models of population genetics. The essence of the problem was well captured by Smouse (1998) in his paper entitled 'To tree or not to tree'. A current illustration of this debate is provided by the controversy surrounding one of the most successful tree-based methods of analysis of phylogeographic data, the nested clade phylogeographic analysis (NCPA, Templeton *et al.* 1995; Templeton 1998, 2001, 2004). NCPA seeks to distinguish

between different historical processes that might have influenced the geographic distribution of haplotypes or lineages relative to higher-level clades. It includes permutation tests of the spatial distribution of these lineages and a protocol for inference of the processes resulting in the particular phylogeographic structure observed (based on an inference key). As noted by Hey & Machado (2003), the promise to identify the relevant processes (isolation by distance, past fragmentation, range expansions ...) has raised considerable interest, because such inferences are generally impossible with any single alternative method. A user-friendly program, called GEODIS, was devised to help researchers implement the NCPA method (Posada *et al.* 2000). As of August 2007, there were over 1600 citations of the original NCPA papers, and about 20% of studies in phylogeography make use of this method (Fig. 1), illustrating its large diffusion in the field.

Alternative methods to NCPA have been proposed, based on analytical models, and these are considered to better take into account the stochasticity inherent in the evolutionary process (Knowles & Maddison 2002; Knowles 2004). Nevertheless, many researchers have continued to use NCPA for single-locus data, pleading for pluralism and pointing to the lack of handy alternatives to analyse the hard-won data (e.g. Weiss & Ferrand 2006), while efforts to include multiple gene trees in NCPA studies have been made (Templeton 2004). Other criticisms of the NCPA method have emerged following the realization that there were deficiencies in the inferences drawn from NCPA for specific historical scenarios or demographic events (e.g. Alexandrino *et al.* 2002; Paulo *et al.* 2002; Masta *et al.* 2003). These findings have led to changes in the methodology or to recommendations of when the approach is more likely to fail (Templeton 2004). A more serious criticism has focused on the practical implementation of the permutation tests used to evaluate the data (Petit & Grivet 2002). Contrary to what is customary when testing the existence of spatial genetic structure by permutation, it is not the populations that are being reshuffled at random in the NCPA procedure but the individuals themselves. As a consequence, processes that can affect local haplotype frequencies, such as bottlenecks (Johnson *et al.* 2007) or mating between relatives, are being confounded with historical processes that have shaped the genetic structure at the range-wide scale, greatly increasing the risk of false-positives (Fig. 2). According to Templeton (2002), the NCPA method would still be valid provided some variation within population exists, but it is unclear how much within-population variation is needed for the method to perform well. Despite these uncertainties, NCPA has continued to attract much interest (Fig. 1).

By far, the largest limitation of NCPA is that few simulation studies have been carried out to validate the inferences made. The only type of validation performed by the proponents of the method was the analysis of a number of cases studies with 'known' a priori expectations (Templeton 1998). Knowles &

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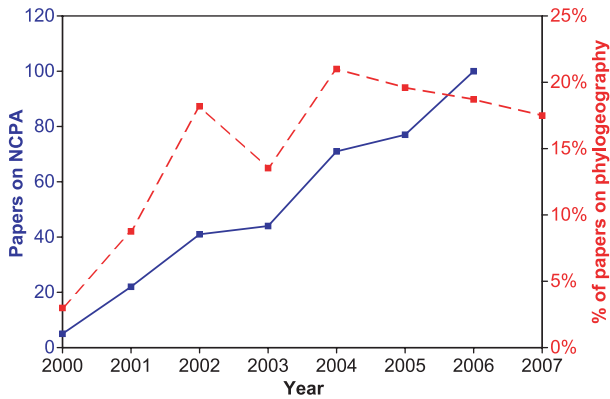


Fig. 1 Trends in the publication of papers using the NCPA method since 2000. As a proxy for papers using the NCPA, we counted papers mentioning the *GEODIS* software. We have also estimated, among all papers dealing with phylogeography, the proportion that uses the NCPA method. Currently, about 20% of phylogeographic studies rely on NCPA.

Maddison (2002) carried out the first (small-scaled) simulation study to examine whether NCPA was able to infer the processes used to simulate the data (few simulations had been used because the results had to be evaluated manually using the inference key). They found that NCPA was misleading in 70% of the cases. However, the significance of these simulations was discussed on the ground that some key aspects of the method had been misrepresented (Templeton 2004).

The new paper by Panchal & Beaumont (2007) describes the automation of the whole NCPA inference process (building the haplotype network, performing the nesting and permutation tests, and applying the inference key) and its evaluation on a larger number of simulated data sets. The description of the corresponding computer program (*ANECA*) is in Panchal (2007); another program called *AUTOINFER* was published nearly at the same time to achieve the same objectives (Zhang *et al.* 2006). After carefully checking that the automated method was working, Panchal and Beaumont simulated, under a panmictic model, 100 genealogies from which they sampled DNA sequences from varying number of populations and individuals. They finally ran *GEODIS* on these simulated samples. The proportion of false-positives reached 76%. This is actually a conservative estimate, since 87% of the simulated data sets have at least one of the two spatial statistics (D_c or D_n) that is significant. The (false) inferences arrived at with their simulated panmictic data sets (restricted gene flow with isolation by distance and contiguous range expansion) are precisely those most frequently found in previous analyses of empirical data sets.

In conclusion, at least three independent analyses (Knowles & Maddison 2002; Petit & Grivet 2002; Panchal & Beaumont 2007) have now shown that the NCPA method returns an excess of false-positives under a number of scenarios. In retrospect, the propensity of NCPA to return too many significant outcomes might have contributed to its appeal among researchers eager to identify statistical support for their interpretation of the data. Given these findings, I suggest that the

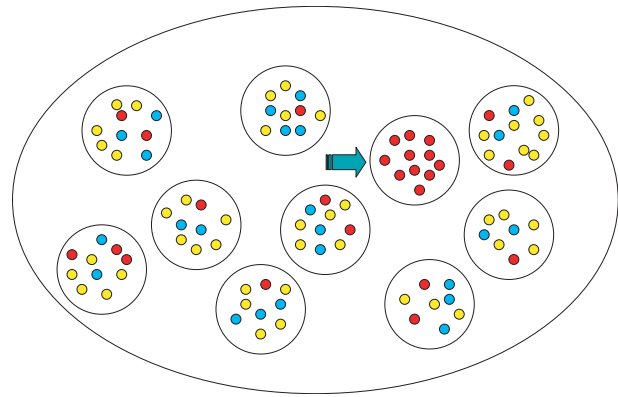


Fig. 2 Local processes can result in false inferences of processes operating at the range-wide scale using the NCPA method. Consider a species that has been randomly mating throughout its history but where one population has recently experienced a bottleneck (marked by an arrow). In such a case, the independent permutation of individuals will result in the detection of a significant spatial structure, and depending on whether the red variant is ancestral or derived, wrong inferences such as range expansion could be reached. The reason is that the sampling of many related individuals in the bottlenecked population effectively results in pseudo-replication for the overall spatial test. Other local processes such as family or clonal structure are also likely to result in false inferences at the range-wide scale. This example illustrates a general problem that still holds for less extreme situations and further shows that increasing sample size within population will only aggravate the problem. Sufficient sampling at the appropriate scale is needed to test for the existence of geographic patterns. The current NCPA permutation procedure, by focusing on individuals instead of populations, is bound to result in over-optimistic tests (Petit & Grivet 2002).

method be no longer used until it has been more thoroughly and critically evaluated, which should be facilitated by the availability of new automated NCPA procedures.

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