

Contrasting Patterns of Differentiation Inferred from Traditional Genetic Markers in the Process of Speciation¹

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ABSTRACT: Populations of *Drosophila serido* from various geographic origins were characterized using four markers: morphology of male genitals, inversions in polytene chromosomes, morphology and heterochromatin of metaphase chromosomes, and reproductive isolation. In all cases the species shows itself to be polytypic, but the geographic distribution patterns vary according to the marker utilized. The validity of the use of these parameters for taxonomic purposes and/or for understanding the mechanisms involved in the differentiation among populations is discussed.

THERE IS NO DOUBT that genetic differences between populations are involved in the process of speciation. However, it is not yet fully clear whether the differences normally detected between species are a cause or a consequence of speciation. Furthermore, it is important to determine what part of the total genome is involved in the first stages of differentiation that are responsible for the appearance of isolation between populations.

Many studies have been done on the enzymatic variability of populations in an attempt to quantify this differentiation by using indexes to measure genetic similarity and distance, mainly between species whose phylogenetic relationships had been previously established using chromosomal and/or morphological traits as markers. The results of studies that attempted to measure or attribute an index to the degree of genetic similarity between species mainly on the basis of enzymatic variability have been quite discordant. Data obtained for different groups of species (*Drosophila*, fish, reptiles, mammals, etc.) have shown that, although they can

be studied, the genetic differences between species in most cases are not correlated with the different degrees of phylogenetic closeness.

Although in most cases the study of different genetic markers does not clarify the genetics of the process of speciation, it has been used as an auxiliary tool in the classification and identification of the various populations of the same species or of different species. The major markers used in these cases have been morphology, chromosome inversions and puff patterns in polytene chromosomes, heterochromatin patterns, banding and structural aberrations in metaphase chromosomes, isozymes, nucleolar organizers, behavioral patterns, reproductive isolation, and ecological differences, among others. It is practically impossible to decide which of these markers is most discriminating, since this seems to vary among the different groups of organisms, in addition to the fact that each marker may differentiate or evolve at different rates.

In view of this problem, over a period of 9 years, we carried out studies on the geographic distribution and differentiation of natural populations of *Drosophila serido*. The results thus far obtained are presented here.

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THE SPECIES *Drosophila serido*

Drosophila serido Vilela & Sene, 1977, is a cactophile species belonging to the *D. buz*

zatii cluster, *mulleri* subgroup, *repleta* group (Wasserman 1982, Wasserman et al. 1983). Our working and collecting area covers a large part of South America and is limited to the north by the Amazon forest, to the west by the Andes Mountains, to the south by Patagonia (Argentina), and to the east by the Atlantic Ocean (Sene et al. 1982). The geographic distribution thus far known shows that the species is detected in any area where Cactaceae are present. Today, cactus plants show discontinuous distribution, which should cause isolation of *D. serido* populations (Vilela 1983, Vilela et al. 1980, 1983). This species does not show ecological specificity, with adults obtained from the following cactus species collected in nature: *Opuntia ficus-indica*, *O. vulgaris*, *Cephalocereus piauhyensis*, *Cereus pernambucensis*, and *Cereus* sp (Pereira et al. 1983).

Populations of different geographic origin were characterized using the following four markers: morphology of male genitals, inversions in polytene chromosomes, morphology and heterochromatin of metaphase chromosomes, and reproductive isolation.

RESULTS OF MARKER ANALYSIS

1. Morphology of Male Genitalia

Five types of aedeagus were detected (G. Silva, personal communication). The geographic distribution of these different types is shown in Figure 1.

2. Chromosome Inversions

The different populations were analyzed by comparing the banding sequence of polytene chromosomes (Tosi 1982). The greatest variability was detected in chromosome II, as is the case for most species in the *repleta* group, to which *D. serido* belongs.

The geographic distribution of the inversions is shown in Figure 2. The populations may be divided into two major groups: one characterized by the presence of the fixed 2W inversion and by the absence of the 2Y inversion, and another in which the 2Y inversion is fixed but the 2W inversion is absent. In

addition to these two large groups, a population (A77) was investigated in which these two inversions were absent. This arrangement detected in population A77 was then considered as a standard for comparison between populations.

Type IV populations having a fixed 2W inversion are polymorphic for inversion 2a. The populations having the 2Y inversion have wide geographic distribution—from the slopes of the Andes in Argentina to the northeast of Brazil. The 2Y populations do not show the 2a inversion but are polymorphic for seven other inversions in chromosome 2, one in chromosome 3, one in chromosome 4, and two in chromosome 5. The distribution of these polymorphic inversions permits subdividing the population bearing the 2Y inversion into three groups, since we considered the difference between populations B58 and A16/B53³ (absence of inversion 2b in the former) to be due to the sampling effect:

- Type I Site B58, inversion 2c/+
Site A16/B53, inversions 2b/+, 2c/+
- Type II Site B31, inversions 2d/+, 2e/+, 5a/+
- Type III Sites B20 and B26, inversions 2f/+, 2g/+, 2h/+, 3a/+, 4a/+, 5b/+

3. Metaphase Chromosomes

Population samples were analyzed for metaphase chromosome structure of neuroblasts from third-instar larvae (Baimai et al. 1983). Differences were detected in the major blocks of constitutive heterochromatin present on the sex chromosomes and/or the sixth chromosome (microchromosome). These chromosomal differences are due largely to the acquisition of extra heterochromatin, although pericentric inversions appear to be responsible for some of the Y chromosome variants.

The cytological evidence demonstrates that populations are far from continuous in

³ A16 and B53 are collections made at the same site at different times.

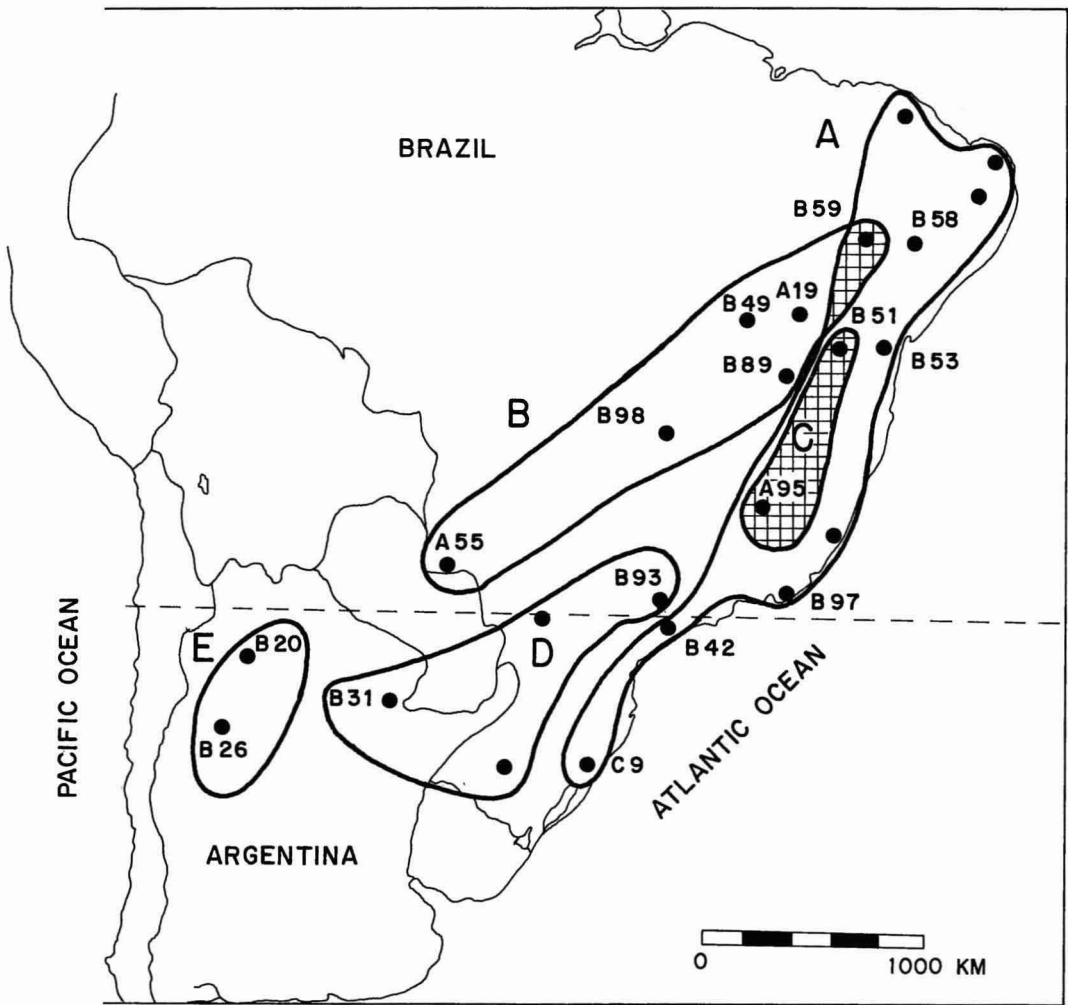


FIGURE 1. Geographic distribution of the five different types (A-E) of the aedeagus of *Drosophila serido*; cross-hatched = sympatric areas (Silva, personal communication).

distribution. They may be divided into six groups, as shown in Figure 3.

4. Reproductive Isolation

Reciprocal crosses involving 14 lines from different geographic regions were performed (Bizzo 1983). In view of the difficulty involved in maintaining this species in the laboratory, each cross was performed by placing five females from one line with two males from another. Copulation occurred in most matings, but the results were very variable. The

following occurrences were noted: (a) copulation, oviposition, and no larvae; (b) unviability of F_1 males in 29 cases and unviability of females in five cases; (c) all F_1 females were fertile; (d) fertile or sterile F_1 males; (e) male sterility was caused by atrophy of the testes and accessory glands, by gonadal dysgenesis, and by azoospermia; (f) in some cases, the offspring of a single cross showed variation from sterile males with fully atrophied testes and azoospermia to normal males.

This gamut of variation in the results is

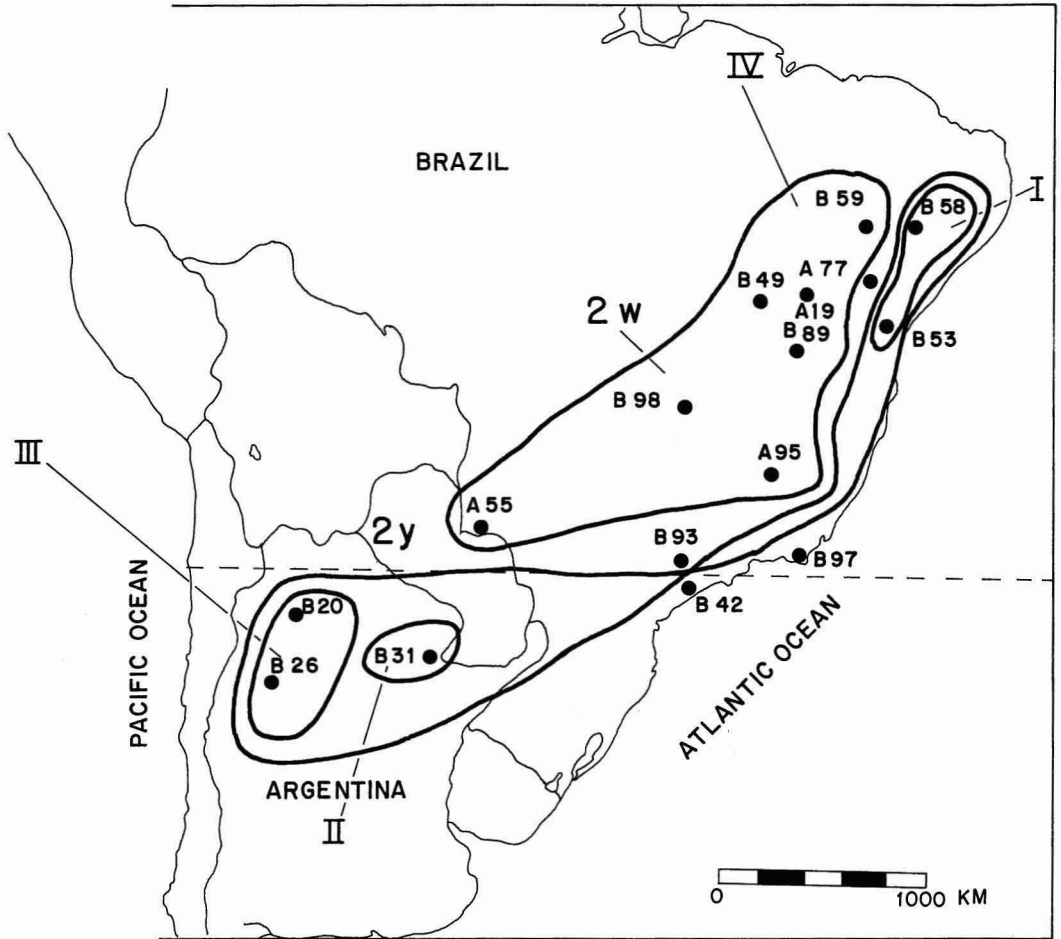


FIGURE 2. Geographic distribution of several polymorphic and monomorphic polytene chromosome inversions of *Drosophila serido*. After Tosi (1982).

made even more complex due to the fact that the different levels of isolation among populations does not obey the geographic distribution that would be expected to occur if differentiation had taken place according to species dispersal throughout the territory. Because of this, it is very difficult to present all the information in a single map. An attempt is made in Figure 4.

GENERAL ANALYSIS OF DATA

A general examination of the figures clearly shows that *Drosophila serido* is at least a polytypic species. At present, we are

not in a position to establish the taxonomic category of each population. This is due to the fact that the geographic pattern varies according to the marker utilized. Thus, five entities are observed if aedeagus morphology is used as a criterion, at least two and at most four when polytene chromosomes are considered, six on the basis of metaphase chromosomes, and at least five on the basis of reproductive isolation.

Some areas are of particular interest because they differ from the others in terms of several markers. The first of these areas is located on the slopes of the Andes in Argentina (B20 and B26). The *Drosophila serido* populations found there show the follow-

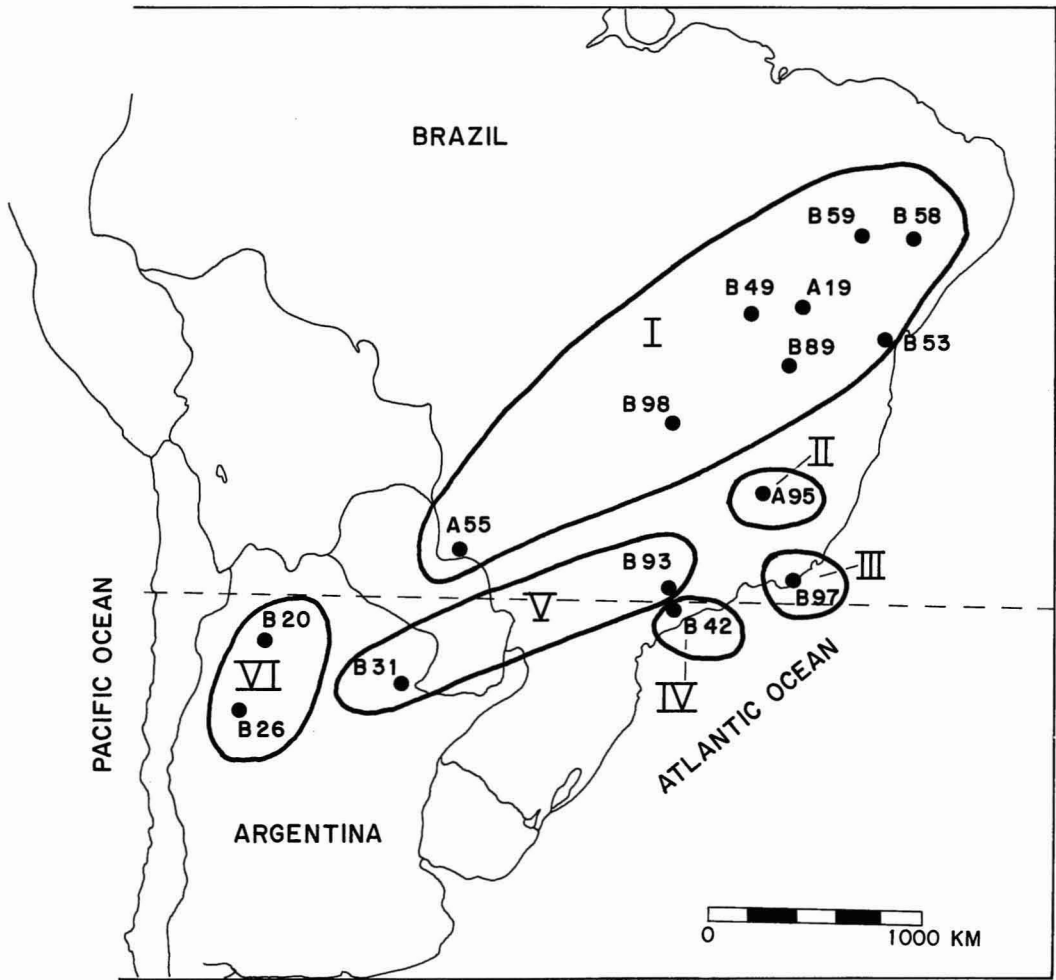


FIGURE 3. Geographic distribution of six different types (I–VI) of metaphase chromosomes of *Drosophila serido*. After Baimai et al. (1983).

ing characteristics, all of which are exclusively detected in these populations: type E aedeagus, type III polytene chromosomes, type VI metaphase chromosomes, and type V isolation pattern. Another such population is that from Serra do Cipó, Brazil (A95), which exhibits type C and type A aedeagus, type II metaphase chromosomes, and type III reproductive isolation. As far as polytene chromosome inversions are concerned, this population has the 2W arrangement found in type IV populations.

In the remaining populations, the situation is quite confused. For example, population B31 has a type D aedeagus, type II poly-

tene chromosome, and type V metaphase chromosomes, but it crosses and produces fertile offspring with population A55, which has type B aedeagus, type IV polytene chromosomes, and type I metaphase chromosomes. Population A19 has a type B aedeagus, type IV polytene chromosomes, and is reproductively isolated from population A16/B53, which has a type A aedeagus and type I polytene chromosomes, although the two populations have identical metaphase chromosomes (type I).

Population B97 has type III metaphase chromosomes, and population B53 has type I metaphase chromosomes. These two popula-

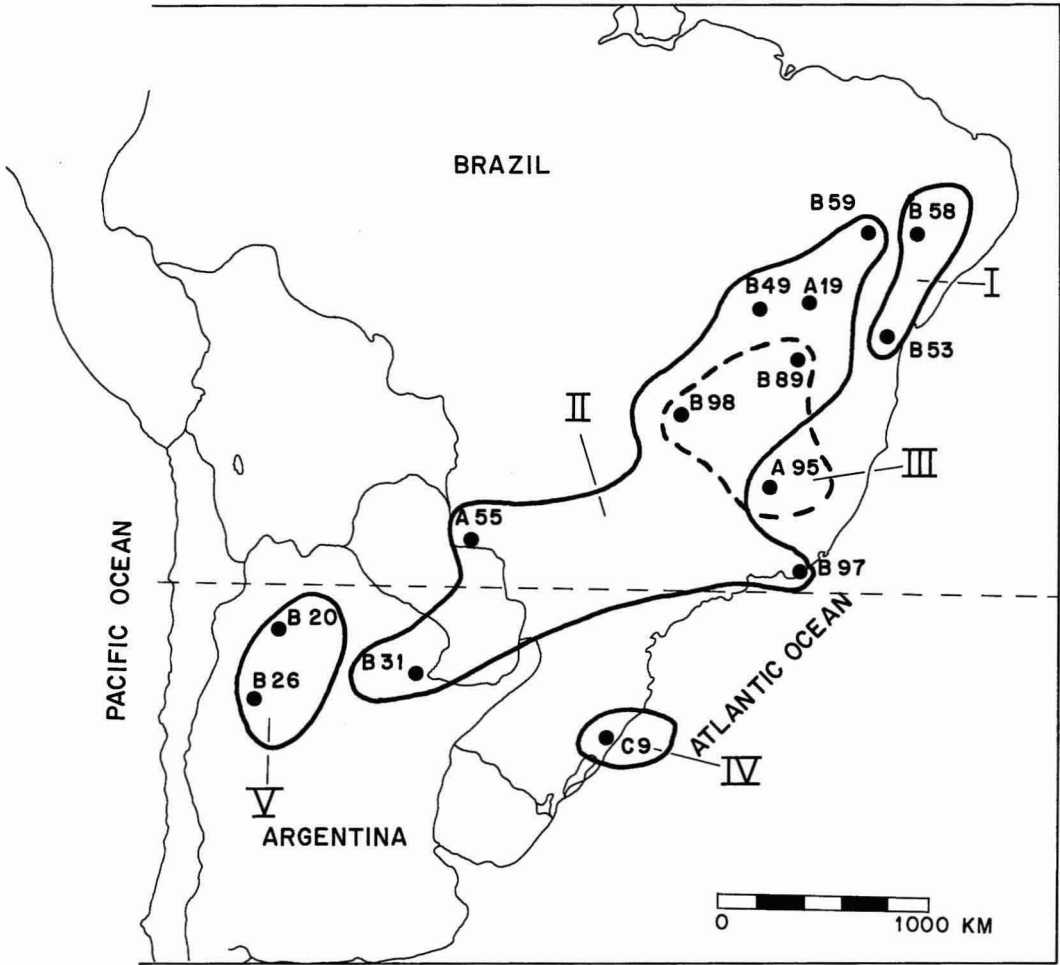


FIGURE 4. Geographic distribution of the reproductive isolation encountered among *Drosophila serido* populations. Adapted from Bizzo (1983).

tions, as well as C9, have type A aedeagus, but they are reproductively isolated among themselves.

Sympatry was detected in two areas in terms of aedeagus morphology: types A and C at sites A95 and B51, and types A and B at site B59.

GEOGRAPHIC AND GEOMORPHOLOGIC CHARACTERISTICS OF AREA STUDIED

The area under study is characterized by the predominance of a tropical climate with relatively small seasonal variations and by

the absence of obvious geographic barriers. The vegetation is diversified, including the following domains: *cerrado* (woody pastures), *caatinga* (a region characterized by stunted thorny vegetation), *chaco* (similar to caatinga), and Atlantic forest. There are also internal forests, araucaria forests, coastal vegetation of the dune type, and rocky fields found on the highlands of central Brazil (Ab'Saber 1977a, Sene et al. 1980).

Paleoclimatic studies carried out in South America (Ab'Saber 1977b, Vanzolini 1981) have shown that cyclic periods of cold and dry climate alternating with periods of hot and humid climate occurred during the

Quaternary period. During the first periods, the vegetation adapted to dry conditions (such as the *caatinga* and *chaco*) expanded, and that adapted to a humid climate (such as forests and *cerrado*) contracted, whereas the situation was inverted during the other periods. Since these periods succeeded each other on the same territory, the consequences of a dry period, for example, are superimposed on the consequences of the preceding dry period. This makes it difficult to determine whether the situations observed today are the consequence of the latest dry period or of a previous one. Available data suggest that the latest dry and cold period occurred 13,000–18,000 years ago, and that vegetation used to be distributed in a manner different from that observed today. For example, the *cerrado* areas were reduced and surrounded with xerophytic formations that permitted the *caatinga* area to be in contact with the *chaco* area. The Amazon and Atlantic forests were reduced to small islands, and the coastal vegetation was widely expanded along the Atlantic coast, forming another link between the *caatinga* and *chaco*. During the next cycle, which was hot and humid, the *cerrado* and forests expanded, thus isolating the areas of dry vegetation. This period may have reached a climax about 2000 years ago, and may still be predominant today.

In this wide territory from the Atlantic coast of the Brazilian northeast to northern Argentina, Cactaceae may be used as markers of the dry areas of today and of the past. We may speculate that the distribution of *Drosophila* species associated with Cactaceae accompanied the expansion and contraction of these plants.

FINAL COMMENTS

As previously stated, it was not our intention to discuss the taxonomic status of the *Drosophila serido* populations studied here. The comments below are made taking into account the following characteristics of these populations: (a) ecologically restricted to areas with cactus plants; (b) females depend on the cladodes of decomposing cacti for

oviposition, which causes the populations of small areas to be submitted to continuous “bottleneck” processes; (c) separate present geographic distribution, a process that must have occurred over the last 13,000 years.

The present results show that different conclusions would be reached on the basis of each marker selected, a fact that obviously casts doubt on the use of markers to study differentiation among populations and speciation.

We may ask the following questions: (a) What processes acted to permit the appearance of differences? (b) Which markers were involved in the primary differentiation process and consequently speciation, and which markers were involved in secondary differentiations? (c) Was the process and/or sequence of events the same for all populations? (d) Did the differences detected today arise over the last 13,000 years when the populations probably separated, or during previous cyclic periods? Obviously, we have no answers to these questions.

In view of the lack of concordance in the differentiation of the markers used, it is impossible to discuss whether the differentiation processes were random or selective. Obviously, lack of concordance is evidence that not all were selective, but only one may have been so and the remaining ones random. We have no way of determining which one of our markers may have been selective. Also, because we used only four of the various markers possible, the marker directly involved in the differentiation process (if it does exist) may not be any of them.

The study of geographic variation of genetic markers has been used extensively to understand the accumulation of variability and polymorphisms in natural populations. The present data cast a doubt on the validity of the use of these parameters for taxonomic purposes and/or for the understanding of the mechanisms involved in the process of speciation.

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