C. R. Biologies 339 (2016) 439–441

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Discussion

The importance of correctly identifying the process responsible for spatial genetic structure in Leopard: A response to McManus and Smuts (2016)

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ARTICLE INFO

Article history:
Received 8 September 2016
Accepted after revision 8 September 2016
Available online 6 October 2016

Keywords:
Response
Leopard
Genetics

ABSTRACT

Microsatellite analyses suggest that spatial genetic structure among six leopard-sampling sites in southern Africa is the result of isolation by distance.

Several factors can influence the accuracy and reliability of geographic population genetic structure inferred from microsatellites [1,2]. One of these is related to the inability of the markers to provide sufficient information to detect population structure [1]. Since the number of markers needed is inversely correlated with the degree of genetic differentiation across populations, the exact sampling parameters for each study are likely to be different. It is furthermore well established that the statistical power of microsatellite analyses can be improved by adding more individuals from each of the sampled populations, by adding additional loci, and or by selecting reasonably variable loci [2]. Since additional loci is only one of the variables to consider, it is no surprise that the primary scientific literature provides ample examples where eight, or even less, variable microsatellite markers provided useful information for the specific questions at hand [3–7]. The claim by McManus and Smuts [8] that "the use of only eight microsatellite markers to report on the contemporary genetic structure strongly limits the statistical accuracy and validity of the results" in Ropiquet et al. [9] is thus merely an opinion without a sound scientific basis.

In Ropiquet et al. [9], variable microsatellite markers were carefully selected based on the information content for leopard as reported in [10], five of the six sampling sites had 18 or more sampled individuals [11], and we believe in concert this contributed to the fact that significant F_{St} values were reported among the sampled leopard populations (Table 2 in [9]). The statistical power of the data used by Ropiquet et al. [9] is best exemplified by a POWSIM

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http://dx.doi.org/10.1016/j.crvi.2016.09.004
1631-0691/

[12] analysis that incorporated the sample sizes used, and the variability of the eight markers. The outcome of this simulation indicated that the statistical power of the data used by Ropiquet et al. [9] had a 100% probability to detect an F_{st} value as low as 0.001.

McManus and Smuts [8] went further to state that Ropiquet et al. [9] firmly concluded that leopards comprise "*a single gene pool*". We found this critique unsubstantiated, since it only partly represents the findings of Ropiquet et al. [9]. In the latter, several clusters were obtained in GENELAND but these made little biological sense since it showed mixing between different sampling regions (suggesting a low level of gene exchange among the GENELAND clusters). The firm conclusions of the results presented by Ropiquet et al. [9] are explicitly stated in the discussion where a synthesis is presented in the first paragraph. It reads "This study represents the most comprehensive analysis of the SGS of southern African leopard. It confirmed that southern African leopards comprise a single population *of distinct geographically isolated groups*". Later in the discussion, Ropiquet et al. [9] again stated that "The two mtDNA markers, which reflect the structure of the female leopard population, *display substantial spatial structure* (Figs. 2 and 3), a result that supports previous findings that female leopards are less mobile than males, or are less frequently translocated". Given the definition of a gene pool (the total number of genes of every individual in an interbreeding population), Ropiquet et al. [9] are correct in stating that there is a single gene pool since the pattern obtained by them reflects a process of isolation by dispersal limitation (IBDL) that lead to a pattern of isolation by distance (IBD) [13,14]. This implies that over shorter geographic distances, individual leopards are interbreeding with each other and in the absence of any other physical barriers to gene flow, the sampled individuals in the Ropiquet et al. [9] study represent a single gene pool (and thus species).

The ill-founded criticisms by McManus and Smuts [8] can have profound implications for leopard conservation. What should conservation managers take from these differences in viewpoint and more importantly, why do they exist? In previous work, McManus et al. [15] argued for the existence of three leopard populations on the southern tip of Africa and proposed human disturbance as the mechanism that created genetic differentiation between the three leopard populations. If this mechanism is correct, it follows logically that human disturbances should then be confined to the sharp boundaries of the parapatric clades they described. In addition, within their larger "central population", no evidence of physical barriers could be detected. We thus regard their explanation for the pattern they obtained unlikely, especially also given the behavioural plasticity of leopards allowing them to persist in highly transformed areas [16,17]. An alternative – and ecologically more plausible – explanation for the genetic structure is put forward by Ropiquet et al. [9] who proposed that the significant population differentiation between sampling sites is due to IBD. This mechanism can indeed explain population differentiation among sampling sites without the assumption that there has to be sharp barriers to gene flow preventing leopards to disperse. In fact, a combined interpretation that can be derived from the mtDNA and nuclear DNA data presented by Ropiquet et al. [9], strongly suggest that male leopards utilize short distance stepwise dispersal to maintain evolutionary connectivity across the region. IBD is an important mechanism [13,14] overlooked by McManus et al. [14]. Not surprisingly, a trend of IBD is also visible in the data presented by McManus et al. [14]. The latter authors reported that the geographically closest clades show the least differentiation (F_{st} value 0.06; $P = 0.072$) and the two individuals representing the more distant northern sampling area show a higher level of differentiation (F_{st} of 0,32; $P = 0.063$). From a scientific viewpoint, however, the lack of an explicit test for IBD, low sample sizes within clades (as low as 2 individuals in one of the comparisons), and the non-significant F_{st} estimates among clades reported by McManus et al. [14] rendered a citation to their work inappropriate.

With the data at hand, we argue again that the genes contained in leopards from different geographical areas in southern Africa are connected to each other in an evolutionary context. The historic pattern determined by the genetic markers employed suggests that small-scale movement occurred between neighbouring populations. The leopards in southern Africa, included in the Ropiquet et al. [9] study, are thus forming one unique gene pool, *and* gene flow decreases with the geographic distance between individuals. Over short time scales (a few generations), distantly sampled individuals are unlikely to show high genetic similarity to each other but over longer time scales (a few hundred generations), long distance stepwise exchange of genetic material maintains a pattern of IBD. There is no convincing conclusive evidence at present to suggest sharp disruptions in leopard gene flow within southern Africa. Since Ropiquet et al. [9] considered mtDNA and nuclear DNA patterns for making their conclusions, the POWSIM analyses support that the data has sufficient statistical power, and a pattern of isolation by distance is congruent with the social behaviour and distribution patterns of leopards [16,17], we strongly advised that leopards should not be translocated over extensive geographic distances.

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