Hybrid Origins: DNA Techniques Confirm that Papilio nandina is a Species Hybrid (Papilionidae)

Authors: Thompson, Martin J., Vane-Wright, Richard I., and Timmermans, Martijn J. T. N.

Source: The Journal of the Lepidopterists' Society, 65(3) : 199-201

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.v65i3.a11

Journal of the Lepidopterists' Society
65(3), 2011, 199–201

HYBRID ORIGINS: DNA TECHNIQUES CONFIRM THAT *PAPILIO NANDINA* IS A SPECIES HYBRID (PAPILIONIDAE)

Additional key words: engrailed, museum collection, mtDNA, *Papilio dardanus*, Princeps

The idea that a significant number of named species will subsequently be discovered to be species hybrids has long been accepted by botanists, even though establishing particular hybrid origins was rarely straightforward. The application of molecular techniques is rapidly changing this field, and clear-cut demonstrations of hybrid origin are now possible (e.g. Siripun & Schilling 2006). However, in a recent survey of "bad species" among butterflies it was estimated that "around 16% of the 440 European butterfly species are known to hybridize in the wild" (Descimon & Mallet 2009: p219). Although hybridisation can lead to new biological species (Kunte et al. 2011), species hybrids clearly represent a taxonomic problem that needs to be addressed by lepidopterists and, as we endeavour to demonstrate here, molecular methods can and surely will play a particularly valuable role in future investigations of putative hybrid origins.

Papilio nandina was described as a new species by Rothschild and Jordan (1901), based on two male specimens caught in East Africa. Butterflies with the *nandina* phenotype are extremely rare in nature but others have been collected since. Initially, Carcasson (1960) considered *P. nandina* to be an aberration of *Papilio phorcas ruscoei* Krüger, 1928. Then, in the 1970s, Carcasson suggested it was a hybrid between the species *Papilio dardanus* Yeats in Brown, 1776, and *P. phorcas* Cramer, 1775 (see Vane-Wright 1976; Vane-Wright et al. 1999; Clarke 1980), with the absence of females possibly explained by Haldane's rule (but see Vane-Wright & Smith 1992). Clarke & Sheppard (1975) and Clarke (1980) succeeded in crossing *P. dardanus* and *P. phorcas* using the hand pairing method (Clarke & Sheppard 1956) and found that the males produced strongly resembled *P. nandina*. It was therefore proposed that wild-caught individuals of *P. nandina* were hybrids and the existence of such a hybrid was (cautiously) given as evidence supporting the grouping of *P. dardanus* and *P. phorcas* as sister taxa.

The present study examines *Papilio nandina* from a molecular perspective. Using the butterfly collections of the Natural History Museum London, we have now extracted DNA from specimens of *P. dardanus* (Voucher BMNH746801-746802, BMNH746805-746806), *P. phorcas* (including a pinned specimen from the 'Majerus Collection'; BMNH808404, BMNH740210-740213), a wild-caught *P. nandina* (collected in 1984 in City Park, Nairobi; Gill, 1986; Figure 4 and accompanying information in Vane-Wright & Smith 1992; BMNH808400), and a 'laboratory' cross of *P. dardanus* and *P. phorcas* (pinned, from the 'Clarke/ Sheppard/ Gill Collection'; Clarke 1991; BMNH808401).

DNA was extracted from single legs according to the protocols of Thomsen et al. (2009). Amplifiable DNA was extracted from all specimens, demonstrating that usable DNA can be obtained from pinned butterfly specimens collected over 25 years ago. Individuals were sequenced for the mitochondrial gene COI (primers HCO2198 and LCO1490; Folmer et al. 1994) and the nuclear gene engrailed (primers: Pd202: 5'-agccagtacacygcaccac-3' and Pd204: 5'-tcyccgatctgmracaccgtctg-3'; 387 base pair amplicon). Sequences were submitted to GenBank (HQ636437-HQ636452).

If the wild-caught *P. nandina* is a hybrid as proposed, then we would expect the nuclear genome to be inherited 50:50 from both *P. dardanus* and *P. phorcas*, and in this respect to be indistinguishable from that of the 'laboratory' hybrid. This is exactly what is found: sequence traces reveal that the *P. nandina* individual carried a distinct *P. dardanus* and a distinct *P. phorcas* allele. Out of 46 polymorphisms revealed in the engrailed sequence, 24 are fixed in both *P. dardanus* and *P. phorcas* with the *P. nandina* individuals displaying the corresponding ambiguity, 6 show shared polymorphisms between *P. nandina* and one of the other species and 16 are uninformative (polymorphic in only one of *P. dardanus* or *P. phorcas*).

The COI fragment from the wild-caught *P. nandina* exactly matches sequences obtained in this study from *P. phorcas* and differs only at a single position from the *P. phorcas* sequence available on GenBank (AF044001; Caterino & Sperling 1999). Mitochondrial DNA is only inherited from the female parent, therefore the wild *P. nandina* specimen is a hybrid between a male of *P. dardanus* and a female *P. phorcas*.

Our results confirm that *P. nandina*, as first suggested by Carcasson, and subsequently demonstrated by Clarke & Sheppard (1975) and Clarke (1980) by breeding experiments, and by Vane-Wright & Smith (1991) on morphological grounds, is not a 'good' species, but represents a species hybrid (Vane-Wright & Smith 1992).

Given that the male parent of the one wild-caught *nandina* that we have been able to analyze must have been *P. dardanus*, it is interesting to note that the males of this species are demonstrably promiscuous with respect to female color patterns, consistent with the amusing comment of W. C. Hewitson following the recognition of female-limited polymorphism in *P. dardanus* (then *P. merope*) by Roland Trimen: "it would require a stretch of the imagination, of which I am incapable, to believe that the *P. Merope* [sic] of the mainland, having no specific difference, indulges in a whole harem of females, differing as widely from it as any other species in the genus." (quoted by Trimen 1874: p140; see Cook et al. 1994 for field observations on mate choice by male *P. dardanus*). Whether or not all wild *nandina* hybrids are sired by *P. dardanus* is a matter for speculation at this point, but it should be remembered that many populations of *P. phorcas* also exhibit female-limited polymorphism—although this is not so spectacular as that seen in *P. dardanus* (Vane-Wright & Boppré 1993).

This molecular investigation demonstrates the value of pinned collections as a source of both morphological and molecular data, and the importance of molecular studies for taxonomy. A similar methodological approach has already been used to investigate another demonstrably hybrid "species", *Erebia serotina* Descimon & de Lesse, 1953, as reported by Descimon & Mallet (2009). The value of the technique presented here lies in the fact that it is not dependent on fresh material; we propose the use of both mitochondrial and nuclear markers on museum material as a valuable tool to assess putative hybrids.

ACKNOWLEDGEMENTS

We thank Blanca Huertas and other members of the Lepidoptera curatorial staff at the NHM for access to the collections, and Steve Collins (ABRI, Kenya) for supplying us with material of *P. dardanus*. We thank Alfried Vogler for supporting the project, and for valuable discussion. We also thank an anonymous reviewer for valuable suggestions regarding the manuscript first submitted. This project was funded by the Natural Environment Research Council (NE/F006225/1).

LITERATURE CITED

CARCASSON, R. 1960. The swallowtail butterflies of East Africa (Lepidoptera, Papilionidae). Journal of the East African Natural History Society, Special Supplement (6): i + 33 pp, 11 pls.

CATERINO, M. S. & F. A. H. SPERLING. 1999. Papilio phylogeny based on mitochondrial cytochrome oxidase I and II genes. Molecular Phylogenetics and Evolution 11: 122–137.

CLARKE, C. A. 1980. *Papilio nandina*, a probable hybrid between *Papilio dardanus* and *Papilio phorcas*. Systematic Entomology 5: 49–57.

———. 1991. The Clarke/Sheppard/Turner genetic collection of butterflies at the Natural History Museum, London. Journal of the Lepidopterists' Society 45: 222–225.

CLARKE, C. A. & P. M. SHEPPARD. 1956. Handpairing of butterflies. Lepidoptera News 10: 47–53.

———. 1975. [Exhibit report]. Proceedings of the Royal Entomological Society of London (C) 39: 39–40.

COOK, S. E., J. G. VERNON, M. BATESON & T. GUILFORD. 1994. Mate choice in the polymorphic African swallowtail butterfly, *Papilio dardanus*: male-like females may avoid sexual harassment. Animal Behaviour 47: 389–397.

DESCIMON, H. & J. MALLET. 2009. Bad species. Pp. 219-249. *In*: J. Settele, T. Shreeve, M. Konvička & H. Van Dyck (eds.), Ecology of Butterflies in Europe. Cambridge: Cambridge University Press.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ & R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

GILL, A. 1986. Some interesting swallowtail butterflies found in City Park. East African Natural History Society Bulletin 1: 2–5.

KUNTE, K., C. SHEA, M. L. AARDEMA, J. M. SCRIBER, T. E. JUENGER, L. E. GILBERT & M. R. KORNFROST. 2011. Sex chromosome mosaicism and hybrid speciation among tiger swallowtail butterflies. PLoS Genetics 7(9): e1002274.

ROTHSCHILD, W. & K. JORDAN. 1901. On some Lepidoptera. Novitates Zoologicae 8: 401–407, 2 pls.

SIRIPUN, K. C. & E. E. SCHILLING. 2006. Molecular confirmation of the hybrid origin of *Eupatorium godfreyanum* (Asteraceae). American Journal of Botany 93: 319–325.

THOMSEN, P. F., S. ELIAS, M. T. P. GILBERT, J. HAILE, K. MUNCH, S. KUZMINA, D. G. FROESE, A. SHER, R. N. HOLDAWAY & E. WILLERSLEV. 2009. Non-destructive sampling of ancient insect DNA. PLoS ONE 4: e5048.

TRIMEN, R. 1874. Observations on the case of *Papilio Merope*, Auct.; with an account of the various known forms of that butterfly. Transactions of the Entomological Society of London 22: 137–153.

VANE-WRIGHT, R.I. 1976. [Abstract] An alternative hypothesis on the evolution of *Papilio dardanus* Brown. Proceedings of the Royal Entomological Society of London C 41(1): 1.

VANE-WRIGHT, R.I. & M. BOPPRÉ. 1993. Visual and chemical signalling in butterflies: functional and phylogenetic perspectives. Philosophical Transactions of the Royal Society (B) 340: 197–205, 2 pls.

VANE-WRIGHT, R. I. & C. R. SMITH. 1991. Phylogenetic relationships of three African swallowtail butterflies, *Papilio dardanus*, *P. phorcas* and *P. constantinus*: a cladistic analysis (Lepidoptera: Papilionidae). Systematic Entomology 16: 275–291.

———. 1992. Occurrence and significance of natural hybrids between *Papilio dardanus* amd *P. phorcas* (Lepidoptera: Papilionidae). Systematic Entomology 17: 269–272.

VANE-WRIGHT, R. I., D. C. RAHEEM, A. CIESLAK & A. P. VOGLER. 1999. Evolution of the mimetic African swallowtail butterfly *Papilio dardanus*: molecular data confirm relationships with *P. phorcas* and P. constantinus. Biological Journal of the Linnean Society 66: 215–229.

MARTIN J. THOMPSON, *Department of Entomology, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.; and Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK; email: m.thompson@nhm.ac.uk,* RICHARD I. VANE-WRIGHT, *Scientific Associate, Department of Entomology, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.; and Durrell Institute of Conservation and Ecology (DICE), School of Anthropology and Conservation, University of Kent, Canterbury CT2 7NE, U.K.; email: dickvanewright@btinternet.com, and* MARTIJN J. T. N. TIMMERMANS, *Department of Entomology, Natural History Museum, Cromwell Road, London SW7 5BD, U.K; and Division of Biology, Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK; email: m.timmermans@nhm.ac.uk.*

Received for publication 23 November 2010; revised and accepted 15 March 2011.