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Dynamics of Chromosome Number Evolution in the *Agrodiaetus phyllis* Species Complex (Insecta: Lepidoptera)

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Abstract—A phylogenetic comparative method was employed to study karyotype evolution in the *Agrodiaetus phyllis* species complex characterized by high variation in haploid chromosome number (from 10 to 134). We found that different phylogenetic lineages of this group have different rates of chromosome-number changes. Chromosome numbers in the complex possess a phylogenetic signal, and their evolutionary transformation is difficult to explain in terms of punctual and gradual evolution.

Keywords: Agrodiaetus, chromosomes, evolution, phylogeny.

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Many organisms are characterized by a stable karyotype and the lack or low level of interspecies variation in chromosome number. The order Lepidoptera is an example. Most of its representatives have the same haploid chromosome number, $n = 31$ (Robinson, 1971). Nevertheless, there are complexes of akin species exhibiting high variation in chromosome number (Yang et al., 1997; Cook, 2003; Lukhtanov et al. 2011). The greatest range of chromosome-number variation (10–134) not associated with polyploidy has been registered in the genus *Agrodiaetus* (Lukhtanov et al., 2005). It is interesting that the whole range is concentrated in a single superspecies complex, *A. phyllis* (Kandul et al., 2007), which is the object of this study.

Chromosome conservatism, on the one hand, and high variability in the chromosome numbers, on the other hand, suggest unequal rates of chromosome number changes and the occurrence of rapid chromosome evolution. However, the mechanisms underlying this process are poorly studied (King, 1993; Lukhtanov et al., 2011). Dramatic differences in karyotypes of closely related species may arise very rapidly due to fixation of multiple chromosome rearrangements (King, 1993). This idea is logically compatible with the model of chromosomal megaevolution (Baker and Bickham, 1980) that describes karyotypic rearrangements taking place in different phylogenetic lineages so rapidly that homologization of rearrangements in various species becomes unfeasible. The latter makes it impossible to reconstruct the phylogeny based on chromosomal traits. Therefore, employing the current phylogenetic terms, it is possible to state that chromosomal changes arising during megaevolution do not carry a phylogenetic signal.

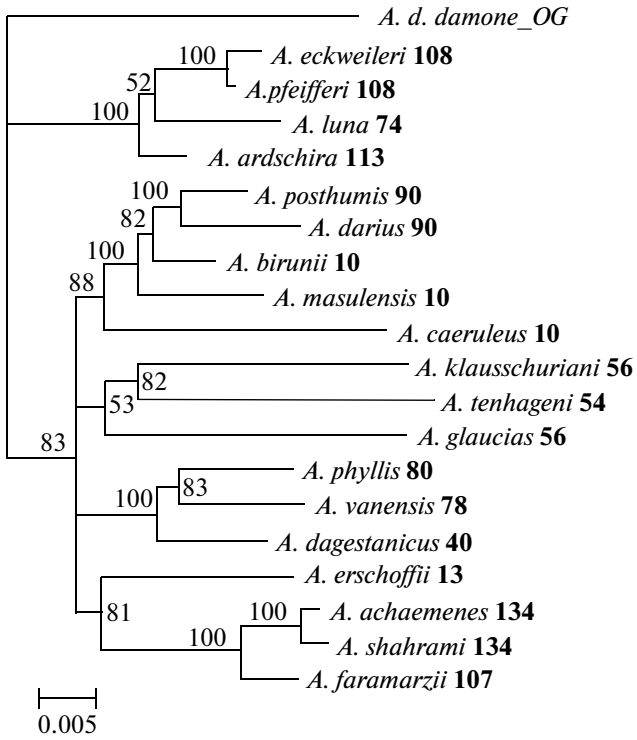
An alternative model is gradual accumulation of chromosomal rearrangements in phylogenetic lineages. The accumulation may occur either uniformly (gradualism) or nonuniformly (punctualism) in time (Lukhtanov et al., 2011). The reliability of the idea of rapid evolution of insect karyotypes has not been verified with the current molecular-phylogenetic approach and is the task of our study.

MATERIALS AND METHODS

The analysis was performed using Pagel's comparative phylogenetic method (Pagel 1999, 2002; Freckleton et al., 2002). Based on phylogenetic data and distribution of traits across the phylogenetic tree, this approach allows to testing of the hypothesis of the presence of the phylogenetic signal and the pattern of trait evolution.

We used original data and others that we had previously published data (Lukhtanov et al., 2005; Kandul et al., 2007) on the haploid chromosome number in all 19 species of the *A. phyllis* species complex (figure). Nuclear and mitochondrial genes were used to reconstruct the phylogeny using Bayesian phylogenetic inference (MrBayes 3.1.2 software (Huelsenbeck and Ronquist, 2001). Bayesian analysis creates a set of probable phylogenetic trees and is appropriate to take into account the hypothetical nature of phylogeny. All sequences were taken directly from the GenBank database (Lukhtanov et al., 2005; Wiemers et al., 2010).

Based on the topology and branch length of 100 constructed phylogenic trees, we calculated the parameters λ and κ using the "Continuous"



The reconstruction was based on mitochondrial (*COI*, *trnL*, *COII*) and nuclear (5.8S *rRNA*, *ITS2*, *28S rRNA*) genes using Bayesian inference (Huelsenbeck, Ronquist, 2001). Posterior probabilities >50% are shown in nodes (*left*); Haploid chromosome numbers are shown close to the species name (*right*). OG—outgroup.

Majority-rule consensus tree of *Agrodiaetus phyllis* species complex.

BayesTraits package (http://www.evolution.reading.ac.uk/BayesTraits.html). The λ parameter shows the phylogenetic signal of the particular trait and may vary from 0 to 1.0. $\lambda = 0$ means that the traits do not possess a phylogenetic signal; i.e., they are distributed across the phylogenetic tree totally randomly and are independent from each other. $\lambda = 1.0$ implies that the traits possess a reliable phylogenetic signal; in theory, the phylogeny may be entirely predicted based on the pattern of trait distribution.

The κ parameter shows the level of the graduality and punctuality in trait changes during phylogenesis. The parameter may vary from 0 to 3. $\kappa = 0$ in the case of absolute punctual evolution in which the traits arise spontaneously and the possibility of the appearance of a new trait in any branch is not a function of time. $\kappa = 1$ ("ideal gradualism") if the rate of evolution is directly proportional to the length of particular branches. $\kappa > 1$ indicates the accelerated evolution of the trait in long branches. $\kappa < 1$ shows that changes mostly occur in short branches; long branches exhibit evolutionary stasis.

The λ and κ values and associated resulting harmonic means for maximal likelihood were estimated empirically. Then, using fixed values of λ (0, 1) and κ (0, 1, 3), we performed modeling of the trait evolution and calculation of harmonic means associated with these fixed values.

The significance of the difference between experimental results and data obtained from modeling was determined using the Bayesian factor (BF). Unlike the common hypothesis in statistical testing based on the comparison with the null hypothesis, the Bayesian factor approach provides direct comparison of two competitive hypotheses. It is considered that the hypothesis associated with higher values of likelihood has is significantly preferable to the hypothesis with less likelihood value, if $BF \geq 2$. This criterion is discussed in greater detail in other papers (Kass, Raftery, 1995; Pagel, 1999).

RESULTS AND DISCUSSION

A consensus phylogenetic tree is shown in the figure. The chromosome number of the *A. phyllis* species complex was mapped on the set of reconstructed trees. BF values are presented in the table.

It was found that the λ parameter significantly differed from 0 and tended to 1. Thus, the chromosome numbers in the *A. phyllis* species complex possess a clear phylogenetic signal; their changes during phylogenesis could not be explained based on the notion of chromosomal megaevolution (Baker, Bickham, 1980).

Different values of the parameter κ characterizing the dynamics of chromosomal rearrangements on branches with various lengths were estimated for various tree topologies. Some topologies had κ values that indicate graduality; others, to the contrary, indicate

Analysis of karyotype evolution in the *A. phyllis* species complex using a comparative phylogenetic method

Parameter	BF	Conclusion
$\lambda = 1.03 \pm 0.06$	2.6 (against $\lambda = 0$)	The model of chromosomal megaevolution is disproved
	2.4 (in favor $\lambda = 1$)	Revealed phylogenetic signal
$\kappa = 0.40 \pm 0.39$	2.2 (in favor $\kappa = 0$)	Accepted hypothesis of punctualism
	1.1 (in favor $\kappa = 1$)	Hypothesis of gradual changes of chromosome numbers is not disproved
	3.5 (against $\kappa = 3$)	Hypothesis of accelerated evolutionary rate in long branches is disproved

Note: Mean values and standard deviations of λ and κ parameters are presented. BF—Bayesian factor (Kass and Raftery 1995; Pagel, 1999).

punctual evolutionary changes of chromosome numbers. Thus, the results of modeling gradual and punctual evolution are inconsistent and an unequivocal interpretation of them still impossible. This conclusion was quite expected. Reconstructed topologies demonstrate that *A. phyllis* complex contains clades of which the representatives have extremely contrasting chromosome variations (figure). The most evident example is the clade (*A. caeruleus* ($n = 10$) + *A. masulensis* ($n = 10$) + *A. birunii* ($n = 10$) + *A. posthumus* ($n = 90$) + *A. darius* ($n = 90$)). However, the group also has clades with relatively small variations in chromosome number—for example, the clade (*A. glaucias* ($n = 56$) + *A. tenhageni* ($n = 54$) + *A. klausschuriani* ($n = 56$)).

Moreover, the approach that we employed disproved the assumption that the evolution of the chromosome number was accelerated on long branches of the tree and became disproportionally rapid compared to other branches. In conclusion, the results we obtained showed dramatic differences in the rate of chromosome-number changes in various phylogenetic lineages of the *A. phyllis* species complex. However, it is difficult to explain the dynamics of these changes using either pure punctual or absolute gradual evolution.

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