



THE ORIGIN OF PROTISTAN SYMBIONTS IN TERMITES AND COCKROACHES: A PHYLOGENETIC PERSPECTIVE

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Received for publication 3 March 1995; accepted 27 February 1996

Abstract — The controversy over whether protist symbionts of *Cryptocercus* and termites were inherited from a common ancestor or transferred secondarily has been long standing. We present here the first phylogenetic test of these hypotheses and show that the transfer hypothesis is better supported.

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Introduction

Ever since the discovery of the same flagellated protists (Oxymonadida and Hypermastigida) in the digestive track of the wood eating cockroach *Cryptocercus punctulatus* and the so-called “lower” termites, there has been a controversy over the evolutionary origin of the symbionts³. Some studies assumed that the protists were inherited in termites and *Cryptocercus* from a common ancestor (Cleveland et al., 1934; Kirby, 1937; Hollande, 1952; Grassé and Noirot, 1959; Honigberg, 1970; Bobyleva, 1975; Nalepa, 1984, 1988, 1994). Others argued that it was possible that the symbionts originated in either the termites or *Cryptocercus* and were subsequently transferred to the other taxon (e.g. Emerson, 1935, reviewing the study of Cleveland et al., 1934). Hennig (1969) supported the transfer hypothesis. If the symbionts were inherited from the common ancestor of both termites and *Cryptocercus*, having the flagellate symbionts would be an ancestral state. Because Hennig considered xylophagy (wood eating) and the associated symbionts to be an evolutionary dead end, he concluded that it was not possible for this to be the ancestral state for the large and diverse group of Blattaria (i.e. cockroaches). Boudreaux (1979) also supported the transfer hypothesis, by proposing that the possibility of the geographical coexistence of the North American *Cryptocercus punctulatus* with the ancestor of the termite *Mastotermes* produced the circumstances that made transfer possible. His argument is weak, however, because he failed to take into account the East Asian *Cryptocercus* and the other termites harboring protist symbionts.

More recently, Thorne (1990, 1991) and Nalepa (1991) debated the relative merits of the transfer and the inheritance hypotheses. Thorne (1990, 1991)

³The term symbionts is used here although the actual relationship between the flagellates and their hosts is questioned (Slaytor, 1992).

observed termites (*Zootermopsis* spp.) killing and consuming *Cryptocercus punctulatus*, and *Cryptocercus* killing and consuming termites. Assuming that the behavior of present-day forms is the same as those in the past, she concluded that gut protists could have been acquired and transferred between termites and *Cryptocercus* or their ancestors under natural conditions. Thorne (1990, 1991) assumes that this transfer is a very old event and that *Cryptocercus* is a "living relict" showing primitive characters reminiscent of earlier cockroaches.

Other authors disagreed with Thorne (Noirot, 1992; Nalepa, 1994; Hahn, 1995). This view seems to be rooted in a preconceived idea of how evolution proceeded—that is, symbiosis is a complex phenomenon and its convergent appearance is consequently hypothesized to be less probable than a secondary loss. Nalepa (1991) considered inheritance of the symbionts as the only probable hypothesis. She argued that Thorne's evidence was too circumstantial and that the ancestors of termites and *Cryptocercus* might not have the same behavioral characteristics as their descendants and that these ancestors did not necessarily encounter each other. She also pointed out that transfer between modern species of related termites was extremely rare and concluded by analogy, that transfer between *Cryptocercus* and termites would have been very unlikely.

The arguments of Thorne (1990, 1991) and Nalepa (1991) are speculative because they are extrapolations of present-day processes. They cannot be endorsed or discarded without further testing. Because the problem of the origin of symbionts is clearly a historical question, inheritance of protozoan symbionts could be studied in a phylogenetic analysis encompassing both insect hosts and protozoan symbionts (Grandcolas and Deleporte, 1992). This phylogenetic perspective was initiated by Hennig (1969) but, curiously, has not been considered since except by Deleporte (1988). Thorne (1990) did not use phylogeny and only mentioned it later (Thorne, 1991) as a framework in which all alternative hypotheses are more or less equally plausible. We argue that the origin of protozoan symbionts in termites and *Cryptocercus* can be more precisely examined using phylogenetic analysis. Although we cannot reconstruct details of the phylogenetic diversification of protozoan symbionts and their insect hosts at this time, we can explore the possibility of testing the alternative hypotheses—inheritance versus transfer—using an available cladistic phylogeny of the blattarian hosts (Grandcolas, 1994).

Phylogenetic Test Of The Inheritance And Transfer Hypotheses

Patterns drawn from phylogenetic analysis can be used to test the validity of evolutionary hypotheses based on the study of processes (Coddington, 1988, 1990; Carpenter, 1989; Brooks and McLennan, 1991; Mayden, 1992; Deleporte, 1993; Grandcolas et al., 1994; Eggleton and Vane-Wright, 1994; Spence and Andersen, 1994). In order to apply such tests to the question of transfer versus inheritance of symbionts in *Cryptocercus* and termites, it is first necessary to have reliable phylogenetic trees for the taxa involved. Although a phylogeny for the symbionts would certainly give insights into the details of the evolution of the symbiosis, the crucial test comes from a phylogeny for the insect taxa. If one agrees that termites, mantids and cockroaches are three monophyletic groups, the inheritance versus transfer hypotheses can be tested by knowing the position of *Cryptocercus*. If there are many nodes between *Cryptocercus* and termites on the phylogenetic tree of these taxa

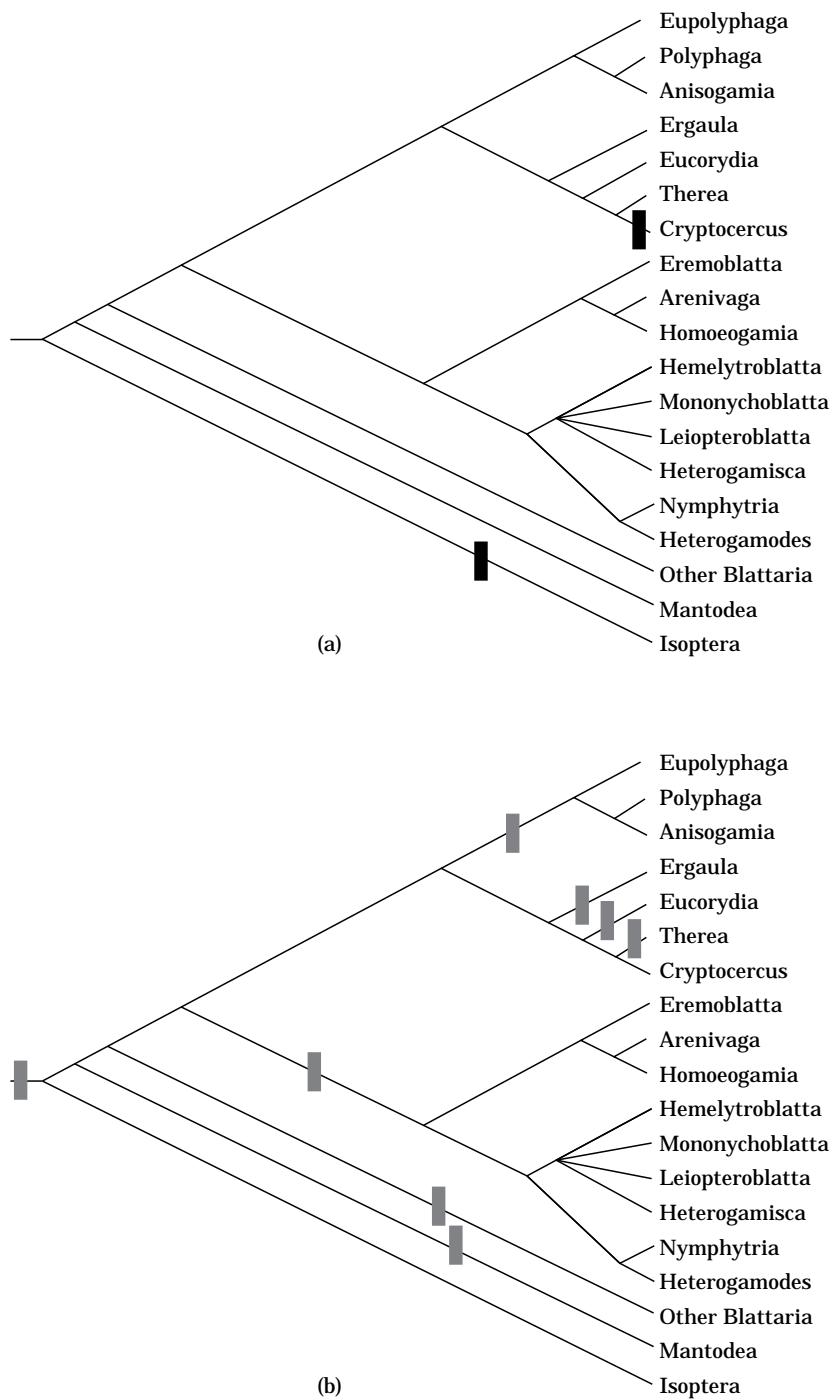


Fig. 1. Hypotheses of the origin of protozoan symbionts on the phylogeny of Polyphaginae cockroaches. (a) The most parsimonious scenario for the transfer hypothesis requires two acquisitions. (b) The most parsimonious hypothesis for the inheritance of symbionts from a common ancestor of termites and cockroaches requires at least seven losses (shaded marks).

(Dictyoptera and Isoptera), symbiont inheritance is not the most parsimonious hypothesis because it implies many losses of symbionts.

Until recently, the phylogenetic position of *Cryptocercus* was not known although it was often hypothesized to be the sister taxon to all other cockroaches (Deleporte, 1988; Thorne, 1991; Thorne and Carpenter, 1992). A recent phylogenetic analysis based on morphological characteristics changes this idea and indicates that *Cryptocercus* is a member of the subfamily Polyphaginae within, not sister-group to, the cockroaches (Fig. 1) (Grandcolas, 1993, 1994, 1996). *Cryptocercus* is not a "living relict" representing the "base" of the Blattaria. More importantly, its position indicates that the inheritance of symbionts is much less parsimonious than the hypothesis of transfer of the symbionts. If the hypothesis of transfer of symbionts is mapped on this tree (Fig. 1a), one transfer event must be hypothesized. If the hypothesis of inheritance of symbiosis from the common ancestor of termites and *Cryptocercus* is mapped on the tree, seven secondary losses of the symbionts must be hypothesized (Fig. 1b). Because it is the less parsimonious hypothesis, the continuity of the symbionts from a common ancestor of cockroaches and termites is less reasonable than the hypothesis of transfer.

The refutation of the hypothesis of symbiont inheritance means that *Cryptocercus* shows traits analogous to those of termites. Flagellate symbionts together with xylophagy and formation of colonies were often seen as necessary ancestral traits for the appearance of eusociality in termites (Cleveland et al., 1934; Myles, 1988; Nalepa, 1984, 1994; Noirot, 1992; Roisin, 1994). *Cryptocercus* appeared to be an interesting model showing these traits. The refutation of the inheritance hypothesis does not preclude the use of *Cryptocercus* as a model **by analogy** for the first steps on the road to eusociality in termites (Nalepa, 1994; Roisin, 1994), provided that one is convinced that symbiosis and xylophagy are two necessary prerequisites for the evolution of eusociality in termites. The possible ancestral occurrence of these traits could be tested when a complete phylogeny of the termites is finally proposed.

Acknowledgements

We are grateful to A. Cloarec, L. Desutter-Grandcolas, A. Gautier-Hion, D. Lipscomb and two anonymous reviewers for their comments on our manuscript. We thank C. Bandi, S. Kambhampati, N. Kristensen, C. Nalepa and L. M. Roth for discussions.

REFERENCES

- BOBYLEVA, N. N. 1975. Morphology and evolution of intestinal parasites flagellates of the far-eastern cockroach *Cryptocercus relictus*. Acta Protozoologica 14: 109–160.
- BROOKS, D. R. AND D. A. McLENNAN. 1991. Phylogeny, Ecology, and Behavior: A Research Program in Comparative Biology. The University of Chicago Press, Chicago.
- CARPENTER, J. M. 1989. Testing scenarios: wasp social behavior. Cladistics 5: 131–144.
- CLEVELAND, L. R., S. R. HALL, E. P. SANDERS AND J. COLLIER. 1934. The wood-feeding roach, *Cryptocercus*, its protozoa and the symbiosis between protozoa and roach. Mem. Amer. Acad. Arts and Sc. 17: 185–342.
- CODDINGTON, J. A. 1988. Cladistic tests of adaptational hypotheses. Cladistics 4: 3–22.

- CODDINGTON, J. A. 1990. Bridges between evolutionary pattern and process. *Cladistics* 6: 379–386.
- DELEPORTE, P. 1988. Etude éco-éthologique et évolutive de *P. americana* et d'autres blattes sociales. Thèse, Université de Rennes I.
- DELEPORTE, P. 1993. Characters, attributes and tests of evolutionary scenarios. *Cladistics* 9: 427–432.
- EGGLETON, P. AND R. VANE-WRIGHT (eds) 1994. *Phylogenetics and Ecology*. Linnean Society Symposium Series No. 17. The Linnean Society of London, Harcourt Brace, London.
- EMERSON, A. E. 1935. Symbiosis between roaches and protozoa. Review of L.R. Cleveland monograph. *Ecology* 16: 116–117.
- GRANDCOLAS, P. 1993. Le genre *Therea* Billberg, 1820: position phylogénétique, nouvelles espèces, répartition et valence écologique (Dictyoptera, Blattaria, Polyphaginae). *Can J. Zool.* 71: 1816–1822.
- GRANDCOLAS, P. 1994. Phylogenetic systematics of the subfamily Polyphaginae, with the assignment of *Cryptocercus* Scudder, 1862 to this taxon (Blattaria, Blaberoidea, Polyphagidae). *Syst. Entomol.* 19: 145–158.
- GRANDCOLAS, P. 1996. The phylogeny of cockroach families: a cladistic appraisal of morpho-anatomical data. *Can J. Zool.* 74: 508–527.
- GRANDCOLAS, P. AND P. DELEPORTE. 1992. La position systématique de *Cryptocercus* Scudder, 1862 et ses implications évolutives. *C.R. Acad. Sc. Paris* 315: 317–322.
- GRANDCOLAS, P., P. DELEPORTE, AND L. DESUTTER-GRANDCOLAS. 1994. Why to use phylogeny in evolutionary ecology? *Acta Oecologica* 15: 661–673.
- GRASSE, P. P. AND C. NOIROT. 1959. L'évolution de la symbiose chez les Isoptères. *Experientia* 15: 365–372.
- HAHN, P. D. 1995. Relatedness asymmetry and the evolution of eusociality. *Sociobiology* 26: 1–32.
- HENNIG, W. 1969. *Die Stammesgeschichte der Insekten*. Senckenberg-Buch. (Translated with additional comments, 1981. *Insect Phylogeny*, John Wiley & Sons).
- HOLLANDE, A. 1952. L'évolution des flagellés symbiotiques, hôtes du *Cryptocercus* et des Termites inférieurs. *Tijd. Entomol.* 95: 81–110.
- HONIGBERG, B. M. 1970. Protozoa associated with termites and their role in digestion. *In: K. Krishna and F. M. Weesner (eds). Biology of Termites.* pp. 1–36.
- KIRBY, H. 1937. Host-parasite relations in the distribution of protozoa in termites. *Univ. Calif. Publ. Zool.* 37: 57–79.
- MAYDEN, R. L. (ed.). 1992. *Systematics, Historical Ecology, and North American Freshwater fishes*. Stanford University Press, Stanford.
- MYLES, T. G. 1988. Resource inheritance in social evolution from termites to man. *In: C. N. Slobodchikoff (ed.). The Ecology of Social Behavior.* Academic Press, San Diego, pp. 379–423.
- NALEPA, C. A. 1984. Colony composition, protozoan transfer and some life history characteristics of the woodroach *Cryptocercus punctulatus* Scudder (Dictyoptera: Cryptocercidae). *Behav. Ecol. Sociobiol.* 14: 273–279.
- NALEPA, C. A. 1988. Cost of parental care in the woodroach *Cryptocercus punctulatus* Scudder (Dictyoptera: Cryptocercidae). *Behav. Ecol. Sociobiol.* 23: 135–140.
- NALEPA, C. A. 1991. Ancestral transfer of symbionts between cockroaches and termites: an unlikely scenario. *Proc. R. Soc. Lond. B* 246: 185–189.
- NALEPA, C. A. 1994. Nourishment and the origin of termite eusociality. *In: J. H. Hunt and C. A. Nalepa (eds). Nourishment and Evolution in Insect Societies.* Westview Press, Boulder, Colorado, pp. 57–104.
- NOIROT, C. 1992. From wood-to-humus-feeding: an important trend in termite evolution. *In: J. Billen (ed.). Biology and Evolution of Social Insects.* Leuven University Press, Leuven, pp. 107–119.
- ROISIN, Y. 1994. Intragroup conflicts and the evolution of sterile castes in termites. *Amer. Nat.* 143: 751–765.
- SLAYTOR, M. 1992. Cellulose digestion in termites and cockroaches: what role do symbionts play? *Comp. Biochem. Physiol. B* 103: 775–784.
- SPENCE, J. R. AND N.M. ANDERSEN. 1994. Biology of water strider: interactions between systematics and ecology. *Ann. Rev. Entomol.* 39: 101–128.

- THORNE, B. L. 1990. A case for ancestral transfer of symbionts between cockroaches and termites. *Proc. R. Soc. Lond. B* 241: 37–41.
- THORNE, B. L. 1991. Ancestral transfer of symbionts between cockroaches and termites: and alternative hypothesis. *Proc. R. Soc. Lond. B* 246: 191–195.
- THORNE, B. L. AND J. M. CARPENTER. 1992. Phylogeny of the Dictyoptera. *Syst. Entomol.* 17: 253–268.