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# The evolution of social behaviour in Blaberid cockroaches with diverse habitats and social systems: phylogenetic analysis of behavioural sequences

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The adequacy and utility of behavioural characters in phylogenetics is widely acknowledged, especially for stereotyped behaviours. However, the most common behaviours are not stereotyped, and these are usually seen as inappropriate or more difficult to analyze in a phylogenetic context. A few methods have been proposed to deal with such data, although they have never been tested on samples larger than six species, which limits their evolutionary interest. In the present study, we perform behavioural observations on 13 cockroach species and derive behavioural phylogenetic characters with the successive event-pairing method. We combine these characters with morphological and molecular data (approximately 6800 bp) in a phylogenetic study of 41 species. We then reconstruct ancestral states of the behavioural data to study evolution of social behaviour in these insects with regard to their social systems (i.e. solitary, gregarious, and subsocial) and diversity of habitat choice. We report for the first time that nonstereotyped behavioural data are adequate for phylogenetic analyses: they are no more homoplastic than traditional data, and support several phylogenetic relationships that we discuss. From an evolutionary perspective, we show that the solitary species *Thanatophyllum akinetum* does not display original behavioural interactions, suggesting phylogenetic inertia of interactive behaviours despite a radical change in social structure. Conversely, the subsocial species *Parasphaeria boleiriana* shows original behavioural interactions, which could result from its peculiar social system or habitat. We conclude that phylogenetic approaches in studies of behaviour are useful for deciphering evolution of behaviour and discriminating between its different modalities, even for nonstereotyped characters.

**KEYWORDS:** phylogenetic inertia – sociality – successive event-pairing method – Zetoborinae.

## INTRODUCTION

Studies of behaviour and phylogenetics have a long and common history (Darwin, 1859; Whitman, 1898; Heinroth, 1909; Lorenz, 1941). After a period of doubt concerning the concept of 'behavioural homology' in the early 1970s (Atz, 1970), the adequacy and utility of

behavioural characters in phylogenetics is now widely acknowledged (Prum, 1990; de Queiroz & Wimberger, 1993; Proctor, 1996; Noll, 2002; Price & Lanyon, 2002; Desutter-Grandcolas & Robillard, 2003; Cap *et al.*, 2008; Pickett, 2010), as long as behavioural characters are clearly defined (Wenzel, 1992). As for morphological and molecular data, behavioural homology hypotheses must be formulated using classical homology criteria (Remane, 1952), and especially the criterion of

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position in behavioural sequences (Baerends, 1958; Robillard *et al.*, 2006; Legendre *et al.*, 2008b), as is the case for DNA or developmental sequences (Edgar, 2004; Schulmeister & Wheeler, 2004; Wheeler *et al.*, 2006; Legendre, Whiting & Grandcolas, 2013).

Ever since the first ethological studies, comparative analyses have focused mainly on highly stereotyped behaviours, such as courtship and ritualized behaviours, which have the advantage of being fixed (or almost fixed) and easy to compare (Hinde & Tinbergen, 1958; Wenzel, 1992). Other behaviours, apparently more variable and difficult to analyze, have, until recently, been neglected in phylogenetic analyses of behaviour (Japyassú *et al.*, 2006; Legendre *et al.*, 2008b; Japyassú & Machado, 2010). Unsurprisingly, all of these recent studies suggest the need for a more accurate coding of behaviour, considering its sequential dimension and focusing on behavioural transitions as phylogenetic characters. However, different methodological coding options and supporting rationales have been proposed. In the present study, we consider the successive event-pairing method (Legendre *et al.*, 2008b), which emphasizes the importance of the too often neglected criterion of position in temporal sequences for assessing behavioural homology. The successive event-pairing method uses transitions among acts in nonstereotyped behavioural sequences as phylogenetic characters. These characters are derived from matrices of transition where each cell is filled with the frequency of a transition between two acts (Legendre *et al.*, 2008b).

In the phylogenetic literature, behavioural acts are often considered in isolation (e.g. the presence/absence of behavioural acts), whereas they are always expressed in a peculiar behavioural context, which is of prime importance as it is well-known among ethologists (cf. displacement and ritualized behaviours; McFarland, 1993). For example, the behavioural act 'leg kick' in mammals could be expressed either in playing, affiliative or agonistic interactions. It is the same behavioural act (the criterion of special quality is fulfilled) but expressed in different contexts (the criterion of position is not satisfied). Similarly, in social insects, 'leg kick after receiving an antennal solicitation' would be a different phylogenetic character than 'leg kick after having been bitten'. The criterion of special quality is again fulfilled but not the position criterion given that 'leg kick' is expressed after two different stimulations and then stands in two different positions. Behavioural studies have shown that these short sequential behavioural patterns within long and nonstereotyped sequences are stable and largely heritable (Gautier, 1974; Grandcolas, 1991; van Baaren *et al.*, 2002, 2003a), which are two qualities required for phylogenetic characters. Therefore, the observation of two different contexts coupled with inheritance

information legitimizes the definition of two different behavioural characters for phylogenetic analysis. Thus, the successive event-pairing method is consistent with two classical homology criteria: the special quality and position criteria (Remane, 1952). Qualitative and quantitative characters can be defined according to the 'presence-absence' of a behavioural character and its frequency of occurrence, respectively. Indeed, frequency information is also stable and largely heritable and can thus be studied in a phylogenetic context, with the main problem being character state delineation (a problem for every kind of continuous phylogenetic data and hence not specific to behavioural data). Consequently, the successive event-pairing method appears to be useful and legitimate for the study of the evolution of behaviour in a phylogenetic context. To date, it has only been tested on a small illustrative data set.

By employing an extended taxonomic sampling, the present study aimed at showing that successive event-pairing not only (1) brings new informative data that are not more homoplastic than 'traditional' (i.e. molecular and morphological) characters, but also (and more evolutionarily relevant) (2) allows detailed hypotheses to be inferred about the evolution of social behaviour. The evolution of social behaviour is a vivid and fertile field encompassing several pivotal evolutionary questions (Hamilton, 1964; Michener, 1969; Trivers, 1971; Lin & Michener, 1972; Wilson, 1975; Emlen, 1982; Thorne, 1997; Lehmann & Keller, 2006; Okasha, 2006; Clutton-Brock *et al.*, 2009; Foley & Gamble, 2009; Garcia & De Monte, 2013). All of these issues would benefit from being considered more often in a comparative framework (Price, Clapp & Omland, 2011).

To achieve our aim, we focus on the evolution of social behaviour in cockroaches. By sampling gregarious, solitary, and subsocial species, we can track behavioural changes possibly associated with changes in social systems. Moreover, given that it is often claimed that environmental conditions strongly influence the evolution of social systems (Jarman, 1974; Slobodchikoff, 1984; Gautier, Deleporte & Rivault, 1988; Slobodchikoff & Shields, 1988; Velicer, Kroos & Lenski, 1998; Foley & Gamble, 2009; Hatchwell, 2009; Kaplan, Hooper & Gurven, 2009), we also track behavioural changes with regard to habitats.

## MATERIAL AND METHODS

### BEHAVIOURAL OBSERVATIONS AND BEHAVIOURAL CHARACTERS

Thirteen species were selected, of which five were previously studied (Grandcolas, 1991; Legendre, Pellens & Grandcolas, 2008a), and behavioural data were

gathered. We focused on the Zetoborinae subfamily, our target group for social evolution that has already been well studied in the field and laboratory (Schal, Gautier & Bell, 1984; Grandcolas, 1998; van Baaren *et al.*, 2002, 2003a; Pellens, Grandcolas & da Silva-Neto, 2002; Pellens, Legendre & Grandcolas, 2007b; Legendre *et al.*, 2008a), and on the Blaberinae subfamily, a closely-related group (Inward, Beccaloni & Eggleton, 2007; Pellens *et al.*, 2007a; Djernaes *et al.*, 2012). Within Zetoborinae, *Thanatophyllum akinetum* Grandcolas, 1991 is solitary (Grandcolas, 1993a) and *Parasphaeria boleiriana* Grandcolas & Pellens, 2002 is subsocial (Pellens *et al.*, 2002). The other Zetoborinae species studied are gregarious (Grandcolas, 1993a; van Baaren *et al.*, 2003b). Within these two subfamilies, only one other genus comprises solitary species (i.e. *Hyporhichnoda*, showing an independent origin of solitariness; Grandcolas, 1998), whereas no other subsocial genus is known. For comparison purposes, we also studied the behaviour of four additional gregarious species from four different subfamilies: the Diplopterinae *Diploptera punctata* Eschscholtz, 1822, the Oxyhaloinae *Nauphoeta cinerea* Olivier, 1789, the Gyninae *Gyna capucina* Gerstaecker, 1883, and the Pycnoscelinae *Pycnoscelus surinamensis* Linnaeus, 1758. We followed the protocol described in Legendre *et al.* (2008a) to record behavioural interactions and then applied the successive event-pairing method (Legendre *et al.*, 2008b), using both qualitative and quantitative data, to derive behavioural characters analyzable in a phylogenetic context.

#### PHYLOGENETIC ANALYSIS

Even though we focus on social evolution in the Zetoborinae and Blaberinae subfamilies, we performed a phylogenetic analysis at the larger scale of the Blaberidae family, as a result of instability in inter-subfamilial phylogenetic relationships in the literature (Maekawa *et al.*, 2003; Inward *et al.*, 2007; Pellens *et al.*, 2007a). The ingroup included 33 species, and 32 identified genera, sampling the 11 known subfamilies in Blaberidae: six Zetoborinae, six Blaberinae, six Panesthiinae, three Oxyhaloinae, three Perisphaeriinae, two Epilamprinae, two Geoscaphinae, two Diplopterinae, one Gyninae, one Pycnoscelinae, and one Panchlorinae. Outgroups comprised three Pseudophyllodromiidae and five Blattellidae, two families previously inferred as being sister-groups to Blaberidae (Grandcolas, 1996; Inward *et al.*, 2007), with *Ectobius sylvestris* Poda, 1761 used as rooting outgroup.

Six molecular markers, for a total amount of approximately 6800 bp, were used to reconstruct Blaberidae relationships: 12S rRNA (approximately 350 bp), 16S rRNA (approximately 510 bp), 18S rRNA

(approximately 1880 bp), 28S rRNA (approximately 2200 bp), cytochrome oxidase subunit I (COI, 1210 bp), and cytochrome oxidase subunit II (COII, 683 bp). We generated 183 molecular sequences, 117 of which are new and specific to the present study, and completed our sampling with 30 sequences downloaded from GenBank (Table 1). Primers and molecular techniques were described in Legendre *et al.* (2008c). In addition, morphological and behavioural data sets were compiled (Table 1). Morphological characters came from Grandcolas (1993b) and include 39 phylogenetically informative characters. Behavioural characters came from dyadic social interactions (Legendre *et al.*, 2008b) and include 213 phylogenetically informative characters. Separate and combined phylogenetic analyses were performed using parsimony and probabilistic methods, which are two approaches with their own strengths and weaknesses (Felsenstein, 1978; Siddall, 1998). The combined phylogenetic matrix is provided in the Supporting information (File S1).

In parsimony, analyses were computed under direct optimization (Wheeler, 1996) with POY, version 4.1.1 (Varón, Vinh & Wheeler, 2010). As in Legendre *et al.* (2008c), ribosomal sequences were preliminary aligned with MUSCLE, version 3.6 (Edgar, 2004) and partitioned according to highly conserved regions (Wheeler *et al.*, 2006), whereas protein coding genes were aligned based on the conservation of codon reading frame in SEQUENCHER, version 4.0 (Genecodes, 1999), and treated as pre-aligned data in POY. Initially, a combined analysis of all molecular data was conducted with 100 replicates and tree bisection-reconnection (TBR) branch swapping, followed by 400 rounds of treefusing (Goloboff, 1999). A long-branch attraction (LBA) artefact was, however, suspected in the resulting topology (see Supporting information, Fig. S1; L = 13401 steps). This artefact hypothesis was tested and corroborated by both a Bayesian analysis (see Supporting information, Fig. S2; ln L = -58999.58; Bergsten, 2005) and a parsimony analysis performed without the long-branched taxon, namely *T. akinetum* (see Supporting information, Fig. S3; L = 13107 steps; Siddall & Whiting, 1999). Indeed, in both cases, the phylogenetic position of *D. punctata* was very different from the one shown in the Supporting information (Fig. S1) (see also Discussion). Consequently, *T. akinetum* molecular sequences were removed before we conducted a sensitivity analysis (gap: transversions: transitions) testing four parameter sets (1:1:1, 2:1:1, 2:2:1, and 4:2:1). Finally, this molecular partition was concatenated with behavioural and morphological data to run a total evidence analysis with 100 replicates and TBR branch swapping followed by 400 treefusing rounds. Nodal support was assessed through partitioned Bremer values (PBV) and Jackknife supports

**Table 1.** List of the 41 species used in this phylogenetic study with information about the data acquired for each taxon (molecules, morphology, and behaviour), including GenBank accession numbers

Species	12S	16S	18S	28S	COI	COII	Morphology	Behaviour
<i>Ancudellia shawi</i>	AB036137	–	AB036189	–	–	AB036097	–	–
<i>Archimandrita tessellata</i>	U17762	U17761	–	–	–	AB014065	–	–
<i>Blaberus discoidalis</i>	EF363290	EF363262	EF363233	<b>KF372445</b>	<b>KF372514</b>	EF363220	<b>X</b>	<b>X</b>
<i>Blattella germanica</i>	EF363293	EF363265	EF363236	EU253813	EU253828	EF363216	–	–
<i>Caecaria crenulata</i>	AB036145	–	AB036197	–	–	AB036103	–	–
<i>Calolampira</i> sp.	–	EU253739	EU253774	EU253816	EU253829	–	<b>X</b>	–
<i>Dendrobliatta</i> sp.	–	<b>KF372480</b>	<b>KF372497</b>	<b>KF372442</b>	<b>KF372515</b>	–	–	–
<i>Diploptera punctata</i>	EF363294	EF363266	EF363237	<b>KF372463</b>	<b>KF372516</b>	EF363211	<b>X</b>	<b>X</b>
<i>Ectobius sylvestris</i>	<b>KF372466</b>	<b>KF372481</b>	<b>KF372498</b>	<b>KF372441</b>	<b>KF372517</b>	–	–	–
<i>Epilampira</i> sp.	EU253698	EU253737	EU253775	EU253815	EU253831	EU253871	<b>X</b>	–
<i>Eublaberus distanti</i>	<b>KF372467</b>	EU367504	EU367508	<b>KF372446</b>	<b>KF372518</b>	<b>KF372541</b>	<b>X</b>	<b>X</b>
<i>Geoscapheus woodwardi</i>	AB036132	AB036178	AB036184	–	–	AB036092	–	–
<i>Gromphadorhina portentosa</i>	EF363297	EF363269	EF363240	<b>KF372464</b>	<b>KF372519</b>	EF363207	–	–
<i>Gyna capucina</i>	<b>KF372468</b>	<b>KF372482</b>	<b>KF372499</b>	<b>KF372447</b>	<b>KF372520</b>	<b>KF372542</b>	<b>X</b>	<b>X</b>
<i>Henshoustedenia</i> sp.	<b>KF372469</b>	<b>KF372483</b>	<b>KF372500</b>	<b>KF372448</b>	<b>KF372521</b>	<b>KF372543</b>	–	–
<i>Isoldata</i> sp.	–	<b>KF372484</b>	<b>KF372513</b>	<b>KF372443</b>	–	–	–	–
<i>Lanxoblattia emarginata</i>	<b>KF372470</b>	EU367505	EU367509	<b>KF372465</b>	<b>KF372522</b>	<b>KF372544</b>	<b>X</b>	<b>X</b>
<i>Laxta</i> sp.	DQ874063	<b>KF372485</b>	<b>KF372501</b>	<b>KF372462</b>	<b>KF372523</b>	<b>KF372545</b>	–	–
<i>Macropanesthia rhinoceros</i>	AB036131	<b>KF372486</b>	<b>KF372502</b>	<b>KF372440</b>	<b>KF372524</b>	–	–	–
<i>Miopanesthia deplanata</i>	AB036151	AB036177	AB036183	–	–	AB036091	–	–
<i>Monastria biguttata</i>	<b>KF372471</b>	<b>KF372487</b>	<b>KF372503</b>	<b>KF372449</b>	<b>KF372525</b>	AB036105	–	–
<i>Nauphoeta cinerea</i>	<b>KF372472</b>	<b>KF372488</b>	<b>KF372504</b>	<b>KF372450</b>	<b>KF372526</b>	<b>KF372546</b>	<b>X</b>	–
<i>Panchlora nivea</i>	EF363301	EF363273	EF363244	<b>KF372451</b>	<b>KF372527</b>	EF363213	–	<b>X</b>
<i>Panesthia eribrata</i>	<b>KF372473</b>	<b>KF372489</b>	<b>KF372505</b>	<b>KF372452</b>	<b>KF372528</b>	<b>KF372548</b>	<b>X</b>	–
<i>Panesthiinae</i>	<b>KF372474</b>	<b>KF372490</b>	<b>KF372506</b>	<b>KF372453</b>	<b>KF372529</b>	<b>KF372549</b>	–	–
<i>Paradicta rotunda</i>	<b>KF372475</b>	<b>KF372491</b>	<b>KF372507</b>	<b>KF372454</b>	<b>KF372530</b>	<b>KF372550</b>	<b>X</b>	<b>X</b>
<i>Parasphaeria boleiriana</i>	EU253699	EU253738	EU253776	EU253817	EU253832	EU253872	<b>X</b>	<b>X</b>
<i>Paratemnopteryx colloniana</i>	DQ874079	<b>KF372492</b>	<b>KF372508</b>	<b>KF372455</b>	<b>KF372531</b>	–	–	–
<i>Phoetalia pallida</i>	<b>KF372476</b>	<b>KF372493</b>	<b>KF372509</b>	<b>KF372456</b>	<b>KF372532</b>	<b>KF372551</b>	<b>X</b>	<b>X</b>
<i>Phortioeca nimbat</i>	–	EU367506	EU367510	<b>KF372457</b>	<b>KF372533</b>	<b>KF372552</b>	<b>X</b>	<b>X</b>
<i>Pseudoglomeris</i> sp.	–	<b>KF372494</b>	<b>KF372510</b>	<b>KF372458</b>	<b>KF372534</b>	<b>KF372553</b>	<b>X</b>	–
<i>Pycnoscelus surinamensis</i>	EF363306	EF363278	EF363249	<b>KF372459</b>	<b>KF372535</b>	EF363208	<b>X</b>	<b>X</b>
<i>Rhabdoblatta formosana</i>	EF363307	EF363279	EF363250	<b>KF372458</b>	<b>KF372535</b>	–	<b>X</b>	–
<i>Salganea esakii</i>	AB036146	AB036180	AB036198	–	<b>KF372536</b>	–	<b>X</b>	–
<i>Schultesia lampyridiformis</i>	EF363308	EF363280	EF363251	<b>KF372460</b>	<b>KF372537</b>	AB007514	–	–
<i>Supella longipalpa</i>	EF363309	EF363281	EF363252	EU253814	EU253834	EF363214	<b>X</b>	<b>X</b>
<i>Thanatophylla akinetum</i>	<b>KF372478</b>	<b>KF372495</b>	<b>KF372511</b>	EU367514 and <b>KF372439</b>	<b>KF372538</b>	–	<b>X</b>	–
<i>Trichoblatta pygmaea</i>	AB036152	AB036182	AB036205	–	–	AB036106	–	–
<i>Xestoblatta cavicola</i>	<b>KF372479</b>	<b>KF372496</b>	<b>KF372512</b>	<b>KF372444</b>	<b>KF372539</b>	<b>KF372554</b>	–	–
<i>Zetobora</i> sp.	EF363311	EF363283	EF363254	<b>KF372461</b>	<b>KF372540</b>	EF363210	<b>X</b>	–

GenBank accession numbers shown in bold represent sequences generated for the present study. –, no data.



(JS). PBV were calculated with TREEROT, version 3 (Sorenson & Franzosa, 2007), using POY implied alignment and 100 replicates, whereas JS were calculated in POY with 50% of characters removed for 1000 replicates.

In Bayesian analyses, molecular data were aligned prior to tree reconstruction with MUSCLE, version 3.6 (Edgar, 2004) and analyzed, with and without morphological and behavioural data. For molecular data, the most appropriate model of evolution was estimated with MRMODELTEST, version 2.3 (Nylander, 2004), using Akaike information criterion (Akaike, 1973; Posada & Buckley, 2004). Phenotypic (i.e. morphology and behaviour) characters were treated as ‘standard’ in MRBAYES, version 3.1.2 (Ronquist & Huelsenbeck, 2003), with the  $M_{kv}$  model of character evolution (Lewis, 2001). Mixed-model analyses allowing different parameter variations between partitions were performed using MRBAYES (Ronquist & Huelsenbeck, 2003; Nylander *et al.*, 2004) and we selected the best-fitting partitioning strategy according to Bayes factor scores (Kass & Raftery, 1995; Nylander *et al.*, 2004). A score above 10 was considered as a strong evidence for the best of two models (Kass & Raftery, 1995). We tested whether we should allow: (1) the nucleotide partition to be subdivided according to the different markers; (2) the phenotypic partition to be subdivided in ‘morphology’ and ‘behaviour’ partitions; (3) a gamma distribution of among rate variation for phenotypic partitions; and (4) different parameters according to nucleotide codon positions for COI and COII.

For Bayesian analyses, which all included *T. akinetum* sequences because probabilistic phylogenetic methods are less sensitive to LBA bias, four chains and two runs were performed for 10 to 60 millions of generations, with trees sampled every 1000–6000 generations. We checked for convergence of our results ensuring that the potential scale reduction factor approached 1.0 for all parameters and that the mean SD of split frequencies converged towards zero. Finally, we used TRACER, version 1.5 (Rambaut & Drummond, 2009), to check that our effective sample size was sufficiently large for a meaningful estimation of parameters and also to assess the burn-in.

#### ANCESTRAL STATE RECONSTRUCTIONS

In parsimony, ancestral states of behavioural data were reconstructed with WINCLADA, version 1.00.08 (Nixon, 1999; command ‘Optimizations’), and we specifically focused on ancestral states tied to social categories and habitats shifts. In other words, we studied nodes where a change in social system or habitat occurs and checked whether these changes take place concomitantly with behavioural transformations. We did not formally reconstruct ancestral

states for social systems because, as a too broadly defined character, it could be potentially misleading (Desutter-Grandcolas & Robillard, 2003; Grandcolas & D’Haese, 2004; Robillard *et al.*, 2006; Grandcolas *et al.*, 2011; Avilés & Harwood, 2012; Doody, Burghardt & Dinets, 2013). However, it might be used in an exploratory approach and would suggest here that a gregarious way of life was the ancestral condition for Zetoborinae. Given that *T. akinetum* and *P. boleiriana* are the only solitary and subsocial Zetoborinae, respectively, changes in social system in Zetoborinae would therefore occur along the terminal branches leading to these two taxa. We thus focused on their autapomorphies. For habitats, data originated from several studies (Grandcolas, 1993a, c; Pellens *et al.*, 2002; Pellens *et al.*, 2007b). Finally, to evaluate whether the behavioural transformations concomitant with social system changes were more numerous than expected by chance alone, we performed a randomization test. For this test, we generated 10 000 randomized behavioural matrices, by permuting each column independently, and parsimoniously reconstructed ancestral states on our optimal topology. We then computed the ratios ‘number of minimal and maximal autapomorphies/tree length’ for both the solitary and subsocial species. We obtained a random distribution for each species and compared the values observed in our case study with these random distributions. This randomization test was designed with the R package phangorn 1.3.0 (Schliep, 2011) and the script is provided in the Supporting information (Doc. S1).

In a probabilistic framework, ancestral state reconstructions were performed under a Bayesian approach with multistate as implemented in BayesTraits (Pagel, Meade & Barker, 2004; Pagel & Meade, 2007). We used the optimal topology and branch lengths from the Bayesian analysis with the best-fitting partitioning strategy (see above). Default parameters were used except for the rate deviation parameter (ratedev = 100) to ensure reaching an adequate acceptance rate (i.e. between 20% and 40%). Ancestral states of behavioural data were reconstructed with a special focus on the most recent common ancestor (MRCA) of the solitary *T. akinetum* and its sister-group on one hand, and of the MRCA of the subsocial *P. boleiriana* and its sister-group on the other hand. Comparing these ancestral states with the character states found in *T. akinetum* and *P. boleiriana*, respectively, allowed us to assess their behavioural autapomorphies. Ancestral state reconstructions were run five times independently with five millions iterations each, and significant reconstructions were assumed when Bayes factor scores exceeded 2 (command ‘fossil’). Similarly, ancestral state reconstructions were performed for habitats, using a hyperprior with an exponential distribution

**Table 2.** List of behaviours, with their abbreviations, displayed by the cockroaches

Behaviours promoting interactions ('positive')	MT	Moving towards the other individual
	AC	Antennal contact with the body of the other individual
	MA	Mutual antennation
	CB	Climbing onto the body of the other individual with one to six legs
Behaviours favouring departure or a break in the interactions ('negative')	PS	An individual puts its pronotum under the other and stands up suddenly
	KL	An individual kicks the other with one leg
	PP	An individual pushes the other with its pronotum
	SP	Stilt posture. An individual rises on its legs
	SA	Stilt posture combined with antennal movement
	SD	Rapidly after a stilt posture, an individual goes down
	BI	An individual bites the other
	SJ	An individual jumps suddenly towards the other
	GD	An individual goes down
	GA	An individual goes down and hides its antennae
	RO	Rotation: turning away from the other individual (without significant displacement of the centre of gravity of the body)
	WD	Withdrawal. An individual moves away from the other
	WP	An individual moves away from the other but stops in proximity
	ES	Escape. An individual moves quickly away from the other
	WA	Sudden withdrawal of the antenna(e)
Behaviours without particular significance	TP	Tilt posture. An individual gives way of legs on its stimulated side
	FP	Freezing posture. An individual does not move at all
	GrA	Grooming behaviour of the antenna
	GL	Grooming behaviour of the leg
	SQ	Stands quiet. The individual stands quiet after a stimulation

(command 'rjhp exp 0 10'). The parameters of the exponential distribution were previously estimated with maximum likelihood analyses (Pagel & Meade, 2007).

## RESULTS

### BEHAVIOURAL OBSERVATIONS

Twenty-four behavioural acts were identified from the dyadic sequences of the different species (Tables 2, 3). Repertoire size ranged from 13 (*D. punctata* and *Paradicta rotunda* Grandcolas, 1992) to 20 acts (*P. boleiriana*), with the solitary species *T. akinetum* having a medium-sized repertoire (17 acts) (Table 3). For the present study, 100 experiments were conducted, which correspond to the recording of 692 behavioural sequences, and 56 experiments originated from Grandcolas' work (1991). On average, behavioural sequences were five to seven acts long but ranged from two to 25 acts. Details about behavioural observations and their analysis out of a phylogenetic context will be provided elsewhere (F. Legendre, A. Gasc, M. Depraetere, P. Deleporte, R. Pellens & P. Grandcolas, unpubl. data).

### PHYLOGENETIC ANALYSIS

The most parsimonious molecular tree in equal weighting is provided in the Supporting information

(Fig. S3; L = 13107 steps). Blaberidae, Oxyhaloinae, and Geoscaphinae were monophyletic, whereas Panesthiinae and Zetoborinae were paraphyletic. Most relationships were stable to parameter changes as revealed by 'Navajo rugs', apart from three successive deep nodes and a subset within Panesthiinae.

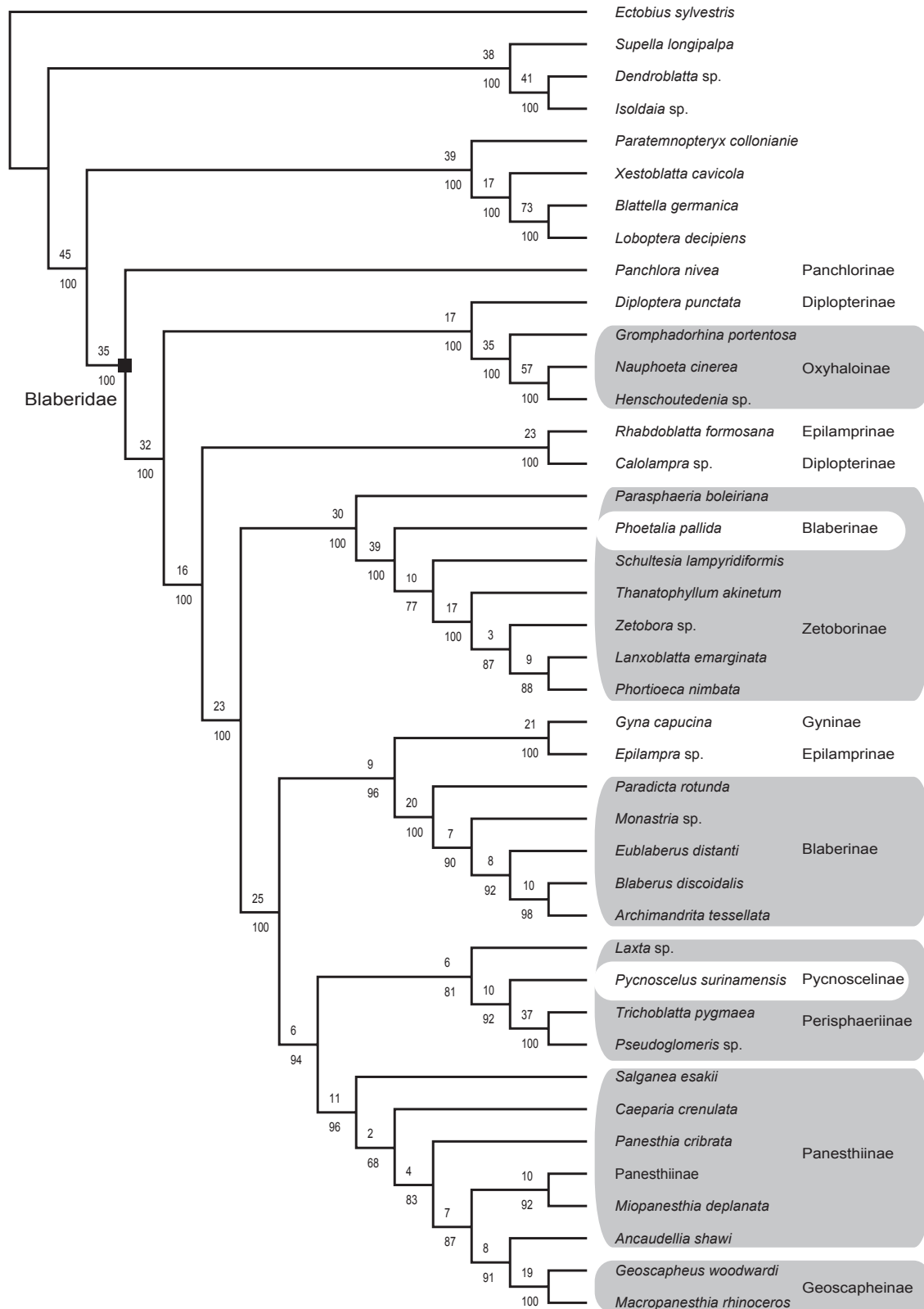
The total evidence analysis resulted in a single most parsimonious topology (L = 13968 steps; Fig. 1) quite similar to the molecular one. Blaberidae were monophyletic with high PBV and JS values (BV = 35; JS = 100). Oxyhaloinae (BV = 35; JS = 100) and Geoscaphinae (BV = 19; JS = 100) were also monophyletic. Panesthiinae were paraphyletic with respect to Geoscaphinae, Perisphaeriinae were paraphyletic as a result of the position of *P. surinamensis* (the single Pycnoscelinae of the study), and Zetoborinae were paraphyletic as a result of the position of the Blaberinae *Phoetalia pallida* Brunner de Wattenwyl, 1865. The nested position of *P. pallida* within Zetoborinae was an unexpected but stable and highly supported result (BV = 30; JS = 100; see also Supporting information, Fig. S3). If *P. pallida* is considered as effectively belonging to Zetoborinae, four out of the eight tested subfamilies were found to be monophyletic (Blaberinae, Geoscaphinae, Oxyhaloinae, and Zetoborinae), two were paraphyletic (Panesthiinae and Perisphaeriinae), and two were polyphyletic (Epilamprinae and Diplopterinae).

**Table 3.** Behavioural repertoires of the 13 species studied

Species/behavioural acts	MT	AC	MA	CB	PS	PP	KL	SP	SA	SD	SJ	BI	GD	GA	TP	FP	RO	WD	ES	WP	GrA	GL	WA	SQ
<i>Blaberus discoidalis</i>	X	X	X	X	X	X		X					X	X	X		X	X		X		X	X	X
<i>Diploptera punctata</i>	X	X	X	X	X	X	X	X		X			X	X	X		X	X		X	X	X	X	X
<i>Eublaberus distanti</i>	X	X	X	X	X	X		X		X			X	X	X		X	X		X	X	X	X	X
<i>Gyna capucina</i>	X	X	X	X	X	X		X					X	X	X		X	X	X		X		X	X
<i>Lanxoblatta emarginata</i>	X	X	X	X	X	X		X	X				X	X	X		X	X		X		X		X
<i>Nauphoeta cinerea</i>	X	X	X	X	X	X		X					X	X	X		X	X	X		X	X		X
<i>Paradicta rotunda</i>	X	X	X	X	X	X		X					X	X	X		X	X		X	X	X	X	X
<i>Parasphaeria boleiriana</i>	X	X	X	X	X	X	X	X	X				X	X	X		X	X		X	X	X	X	X
<i>Phoetalia pallida</i>	X	X	X	X	X	X	X	X	X				X	X	X		X	X		X	X	X		X
<i>Phortioeca nimbata</i>	X	X	X	X	X	X		X	X	X			X	X	X		X	X		X	X	X		X
<i>Pycnoscelus surinamensis</i>	X	X	X	X	X	X		X	X	X			X	X	X		X	X		X	X	X		X
<i>Schultesia lampyridiformis</i>	X	X	X	X	X	X	X	X	X			X	X	X	X		X	X	X		X	X		X
<i>Thanatophyllum akinetum</i>	X	X	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X		X	X		X

Abbreviations of behavioural acts are as defined in Table 2.





**Figure 1.** Most parsimonious tree found in the analysis combining molecules (without *Thanatophyllum akinetum* sequences), morphology and behaviour (L = 13968; CI = 0.40; RI = 0.38). All characters and transformations were equally weighted. Numbers above and below branches are Bremer and Jackknife support values, respectively.

**Table 4.** Phylogeny-related statistics for molecular, morphological and behavioural partitions in the parsimony total evidence analysis

Partitions	CI	RI	$\Sigma$ Bremer	% Bremer (%)	NI	nPBV
Molecules	0.37	0.31	658	82.1	1848	4.4
Behaviour	0.52	0.36	123	15.4	213	7.2
Morphology	0.64	0.62	20	2.5	39	6.4
Total	0.38	0.32	801	100.0	2100	–

CI, consistency index; RI, retention index; NI, number of informative characters; for each partition  $i$ : % Bremer $_i$  =  $\Sigma$  Bremer $_i$ / $\Sigma$  Bremer $_{TOTAL}$  and nPBV $_i$  = (% Bremer $_i$ /NI $_i$ ) $\times$ 100. nPBV is a normalized PBV.

Overall, the optimal topology was well supported, with approximately 80% of the nodes having JS and PBV support above or equal to 90 and 10, respectively.

Consistency and retention indices of the behavioural partition were similar to those of the molecular partition and its normalized PBV showed that, with morphology, behaviour brought a strong phylogenetic signal (Table 4).

In a Bayesian framework, a general time reversible model with a proportion of invariant sites and a gamma distributed rate variation among sites was selected for molecular data as the best fit of the models investigated (model GTR + I +  $\Gamma$ ). As for the best-fitting partitioning strategy, the results are summarized in Tables 5, 6. It shows that allowing each marker to have its own model, taking into account nucleotide positions in protein-coding genes, and adding a gamma-distributed rates across characters for phenotypic data was the best-fitting strategy (i.e. model with 12 partitions). We found the monophyly of the Blaberidae family and of Oxyhaloinae and Geoscapheinae subfamilies with maximal posterior probabilities (see Supporting information, Fig. S4; ln L = -62138.00). Similar to the parsimony analyses, Zetoborinae, including *P. pallida*, and Blaberinae subfamilies were monophyletic as well. The main disagreement between both approaches concerned deeper relationships, most of which are poorly supported. Indeed, the Bayesian topology showed several short internal branches within Blaberidae. Similarly, sensitivity analysis revealed that the less stable nodes corresponded to inter-subfamilial relationships (see Supporting information, Fig. S3). This disagreement between both methods, however, had no consequence on our hypotheses on the evolution of social behaviour given that, in both cases, Zetoborinae and Blaberinae were closely related.

#### ANCESTRAL STATE RECONSTRUCTIONS: BEHAVIOUR AND SOCIALITY

In parsimony, unambiguous ancestral state reconstructions (Fig. 2) revealed that behavioural autapomorphies range from 0 to 66. The solitary species *T. akinetum* had 19 autapomorphies, whereas the subsocial species *P. boleiriana* had 66 autapomorphies.

*Thanatophyllum akinetum* autapomorphies (Table 7) comprised the presence of eight and the absence of three qualitative behavioural characters. The eight other autapomorphies were quantitative characters (frequencies of behavioural transitions; Legendre *et al.*, 2008b). Seven out of the eight autapomorphies exhibited by this solitary species were behaviours that tend to limit interactions between individuals (e.g. ‘bite’, ‘leg kick’, ‘escape’) (Table 7).

Some *P. boleiriana* autapomorphies are listed in Table 7. Out of the 59 qualitative behavioural characters coded present for this species, 27 were behaviours involving avoidance reactions, 11 were behaviours involving aggressive reactions, and only nine were behaviours involving ‘positive’ reactions to solicitations, with the 12 remaining characters being without obvious signification. *Parasphaeria boleiriana* had the highest number of autapomorphies, although it was also the species with the largest repertoire, such that this result could be expected if we suspected a correlation between repertoire richness and number of autapomorphies. This correlation was tested and rejected ( $r = 0.439$ ,  $P > 0.10$ ; data not shown).

Ancestral state reconstructions in a Bayesian framework gave similar behavioural autapomorphies for both species as the parsimony reconstructions, despite topological differences. Indeed, 75% of *T. akinetum* and *P. boleiriana* autapomorphies were shared in both paradigms. Within the remaining 25%, only three character states were significantly supported as autapomorphies (i.e. 4%; Bayes factor > 2). Note that, out of the 85 (13 + 72) qualitative behavioural characters inferred as autapomorphies for these two species, 29 were only exhibited either by *T. akinetum* or *P. boleiriana* (i.e. consistency index of 1).

Finally, the solitary species *T. akinetum* did not exhibit more behavioural autapomorphies than expected by chance alone (randomization tests:  $P = 0.962$  and  $P = 0.986$  for minimal and maximal changes, respectively), whereas the subsocial species *P. boleiriana*

**Table 5.** Summary of the Bayesian analyses showing the model used for each analysis and the resulting log likelihood

Analyses	Phenotypic data model			Molecular data model						ln L
	Partitions	Morphology	Behaviour	12S	16S	18S	28S	COI	COII	
A	2	Mk		GTR + I + $\Gamma$						-66346.56
B	3	Mk	Mk	GTR + I + $\Gamma$						-66306.40
C	8	Mk	Mk	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	-63386.65
D	8	Mk + $\Gamma$	Mk + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	GTR + I + $\Gamma$	-63369.05
<b>E</b>	<b>12</b>	<b>Mk + <math>\Gamma</math></b>	<b>Mk + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>GTR + I + <math>\Gamma</math></b>	<b>-62138.00</b>

GTR, general time reversible model; I, proportion of invariant sites;  $\Gamma$ , gamma rate variation; pos, different parameters according to codon positions; Mk, Markov k model; ln L, arithmetic mean of the log likelihoods. The best fitting partition strategy is shown in bold.

**Table 6.** Summary of the Bayesian analyses showing Bayes factor scores used to assess the best fitting partition strategy

Analyses	Bayes factor	Interpretation
A versus B	80.32	Significant = allow the subdivision of phenotypic data
B versus C	5839.50	Significant = allow the subdivision of molecular data
C versus D	35.20	Significant = add a gamma law to the Markov k model
D versus E	2462.10	Significant = allow subdivision of coding gene according to nucleotide positions

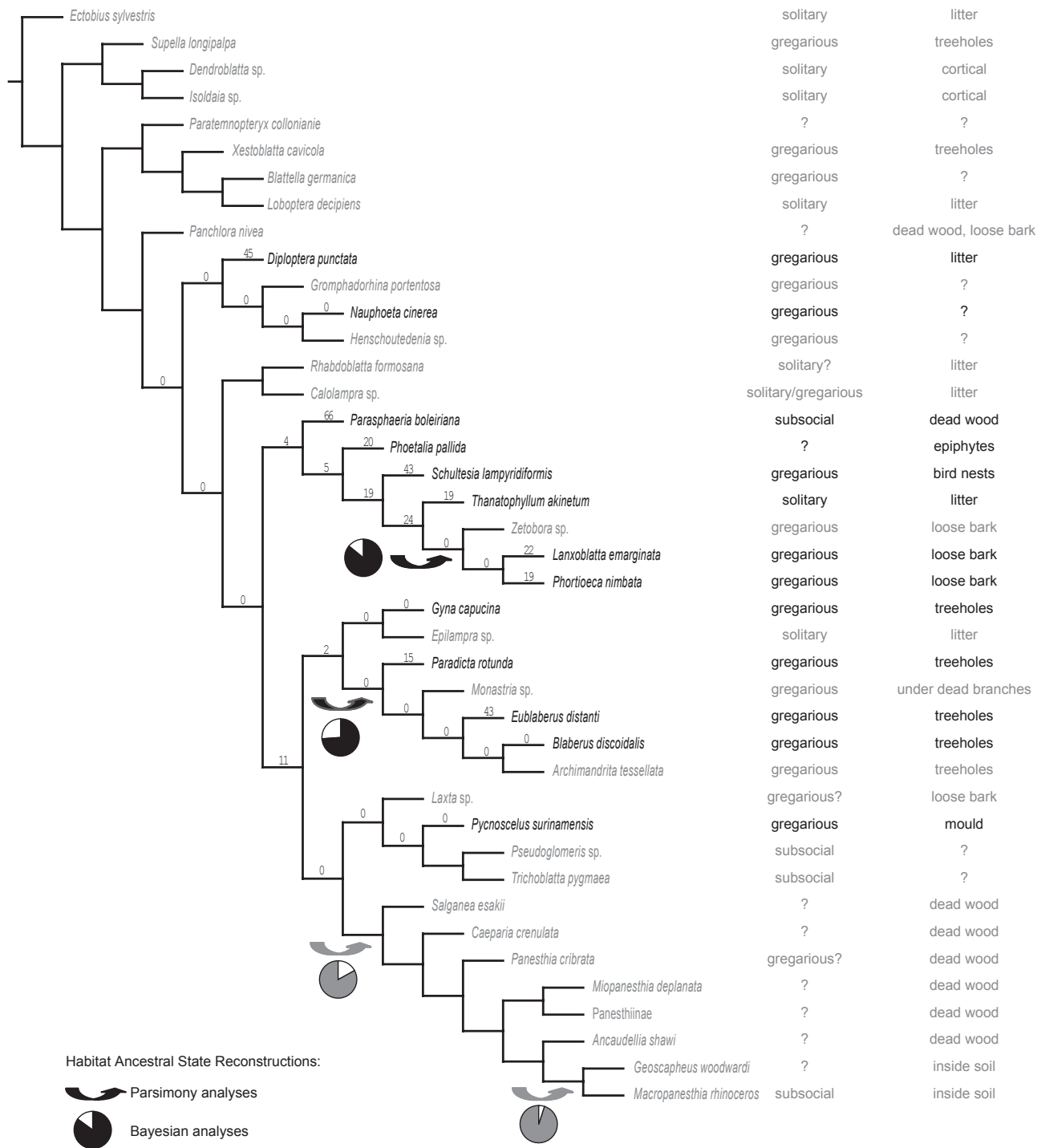
Scores above 10 are significant.

did (randomization tests:  $P < 0.001$  in both cases) (Fig. 3).

#### ANCESTRAL STATE RECONSTRUCTION: BEHAVIOUR AND HABITATS

Unambiguous behavioural character optimization was investigated with regard to habitats. Even though 'habitat' is not a very precisely defined character, it can be optimized a posteriori to tree reconstruction, and behavioural changes occurring concomitantly with some habitat change can be inferred. Some internal nodes had an unknown habitat state as a result of a high diversity in habitats and a few missing data, although two of them were studied within Zetoborinae and Blaberinae: the nodes related to 'treeholes' and 'loose bark' habitats (Fig. 2). No unambiguous behavioural change was associated with either habitat shift. Note that, in both cases, the plesiomorphic habitat was ambiguous. Two other habitat shifts were inferred for Panesthiinae and Geoscaphinae (Fig. 2) but, because no species from these subfamilies was ethologically studied, we do not discuss these habitat shifts any further.

Behavioural comparisons between species living in similar habitats were also performed. The sister species *Lanxoblatta emarginata* Burmeister, 1838 and *Phortioeca nimbata* Burmeister, 1838 both live under loose bark, are very similar morphologically and share similar repertoires (Table 3). However, each had peculiar behavioural autapomorphies (Table 8) involving proportionally more 'positive' reactions for *P. nimbata* (approximately 43%) than for *L. emarginata* (approximately 33%). Species living in treeholes were even more behaviourally diverse. *Eublaberus distanti* Kirby, 1903 had 43 behavioural autapomorphies, among which several involve very



**Figure 2.** Unambiguous ancestral state reconstructions of behavioural data on the most parsimonious tree with information about habitats and social systems. The species whose behaviour was studied are shown in black. Numbers on branches represent the number of unambiguous behavioural changes. Large arrows point at nodes with habitat changes in parsimony. Pie diagrams illustrate the probability associated with these habitat changes in Bayesian reconstructions.

**Table 7.** List of behavioural autapomorphies of the solitary and subsocial species

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Autapomorphic behavioural transitions coded for *Thanatophyllum akinetum*: a solitary species

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Present	<b>MT (moving toward)/BI (bite)</b> <b>AC (antennal contact)/KL (leg kick)</b> KL (leg kick)/AC (antennal contact) <b>SA (stilt + antennation)/WP (light withdrawal)</b> <b>BI (bite)/ES (escape)</b> RO (rotation)/KL (leg kick) <b>WP (light withdrawal)/GA (go down and hide antennae)</b> <b>SQ (stands quiet)/GA (go down and hide antennae)</b>
Absent	AC (antennal contact)/SP (stilt posture) SP (stilt posture)/WD (withdrawal) <b>WP (light withdrawal)/WD (withdrawal)</b>

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The most relevant autapomorphic behavioural transitions coded for *Parasphaeria boleiriana*: a subsocial species

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Present	<b>MT (moving toward)/TP (tilt posture)</b> MT (moving toward)/GA (go down and hide antennae) <i>PP (push with pronotum)/PP (push with pronotum)</i> <b>PP (push with pronotum)/KL (leg kick)</b> <b>PP (push with pronotum)/TP (tilt posture)</b> <b>KL (leg kick)/PP (push with pronotum)</b> <b>KL (leg kick)/TP (tilt posture)</b> <b>SP (stilt posture)/GD (go down)</b> <b>SP (stilt posture)/WP (light withdrawal)</b> <b>SP (stilt posture)/ES (escape)</b> <b>TP (tilt posture)/PP (push with pronotum)</b> <b>GD (go down)/KL (leg kick)</b> <b>ES (escape)/ES (escape)</b>
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Autapomorphies found only in parsimony analysis are shown in normal type. Autapomorphies found only in Bayesian analysis are shown in italics. Autapomorphies found in both parsimony and Bayesian analyses are shown in bold.

aggressive behaviours (jump – SJ; pronotal attack – PS; push with pronotum – PP) (Table 8), whereas *Blaberus discoidealis* Serville, 1838 showed no unambiguous behavioural autapomorphy. As for *P. rotunda*, it had few autapomorphies ( $N = 15$ ), most of which were the ‘absence’ of some behavioural transitions.

## DISCUSSION

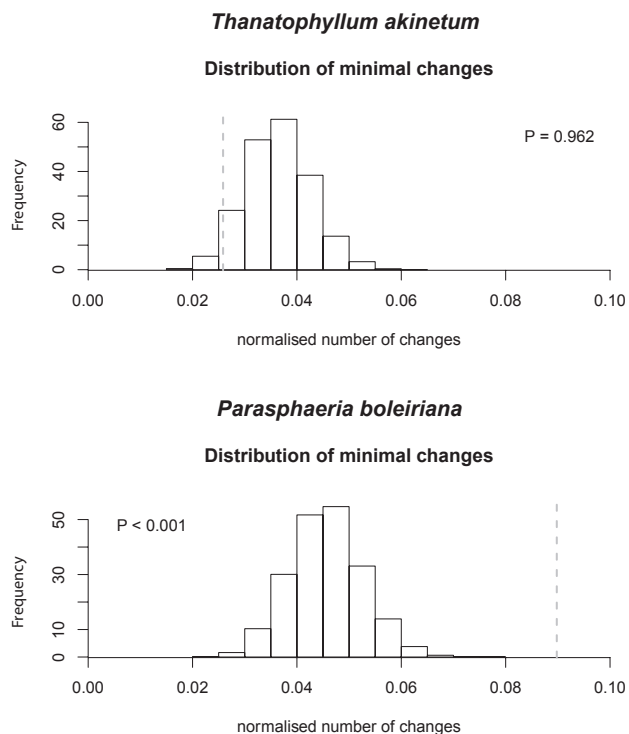
### BEHAVIOURAL DATA IN PHYLOGENETICS AND THE SUCCESSIVE EVENT-PAIRING METHOD

When the successive event-pairing method was proposed, it was conceptually defined and applied to an illustrative case study involving five species (Legendre *et al.*, 2008b). In the present study, we extend this dataset and show that the method remains operational and efficient with this larger sampling. First, behavioural characters defined with the successive event-pairing method are no more homoplastic than traditional characters as revealed by their consistency and retention indices. Second, these behavioural data

bring a substantial signal for tree reconstruction with a high normalized Bremer support. Thus, the present study shows once again that behavioural characters can be valuable phylogenetic characters (Wenzel, 1992; de Queiroz & Wimberger, 1993; Proctor, 1996; Brooks & McLennan, 2002; Cap, Aulagnier & Deleporte, 2002). This property has already been mentioned for stereotyped behaviours (de Queiroz & Wimberger, 1993; Robillard *et al.*, 2006) but never for characters derived from nonstereotyped behavioural sequences such as those used here.

Most interestingly, the successive event-pairing method allows testing and proposing detailed hypotheses in the evolution of behaviour as exemplified here with the social behaviour of Zetoborinae and Blaberinae cockroaches. We focused on a few species to assess their behavioural peculiarities in a phylogenetic context. These species were selected either given their social systems (the solitary species *T. akinetum* and the subsocial species *P. boleiriana*) or their habitats (species living in treeholes and under loose bark).





**Figure 3.** Result of the randomization test showing that the solitary species *Thanatophyllum akinetum* (top) has few behavioural autapomorphies. The random distribution (histogram) reveals that a higher number of autapomorphies than the one observed for *T. akinetum* (grey dash line) might be reached by chance ( $P = 0.962$ ). By contrast, the probability that the subsocial species *Parasphaeria boleiriana* (bottom) shows so many behavioural autapomorphies by chance is extremely low ( $P < 0.001$ ). For both species, the results are similar when maximal number of changes are considered ( $P = 0.986$  and  $P < 0.001$ , respectively; data not shown).

#### SOLITARINESS, SUBSOCIALITY, ADAPTATION, AND PHYLOGENETIC INERTIA

The shifts from gregariousness to solitariness or subsociality imply the loss or gain of key features ('tolerance to conspecifics' and 'parental care', respectively) and related behaviours (Wilson, 1971). In this context, we would expect either numerous behavioural changes concomitant to these shifts in social systems or at least a few but highly significant behavioural changes in species interactions.

Because aggregation behaviour can be explained in terms of interactions between individuals (Jeanson *et al.*, 2005), solitariness could thus be explained by specific behaviours, including aggressions, which would favour spacing among conspecifics (King, 1973). Previous studies (Grandcolas, 1991; Legendre *et al.*, 2008a) showed that the solitary species *T. akinetum* has a lower tolerance to conspecifics compared to

closely-related gregarious species, with individuals passively spacing themselves out instead of remaining aggregated. However, ancestral state reconstructions of behavioural data show that *T. akinetum* has only 19 behavioural autapomorphies, which is similar to the mean number observed for all the species studied, and that its repertoire does not include additional aggressive behavioural acts. Therefore, *T. akinetum* has not evolved solitary habits by deeply changing its behaviour, and aggressive behaviours by themselves cannot explain its solitary way of life. Previous studies already failed to demonstrate a causal link between aggressiveness and solitariness in cockroaches in broad but nonphylogenetic species comparisons (Breed, Hinkle & Bell, 1975; Bell *et al.*, 1979; Gorton & Gerhardt, 1979). Given the lack of significant behavioural differentiation, evolution toward solitariness in *T. akinetum* would have involved a common ancestral repertoire used in a slightly different way, and not have resulted from dramatic changes in behavioural interactions. A close look at behavioural transitions shows that most of *T. akinetum* autapomorphies are related to acts limiting interactions and that dyadic interactions were less often initiated with the act 'moving toward a conspecific' than for other species, but instead resulted generally from fortuitous encounters. In this species, individuals do not refuse interactions (Legendre *et al.*, 2008a) but they do not look for them either. Thus, solitariness would be explained by a simple lack of intraspecific attraction, except for reproduction purposes. In the field, individuals disperse away immediately after hatching so that conspecific interactions are rare, as already suggested by a 2-month field study (Grandcolas, 1993a). Given such dispersal habits, behavioural acts favouring social interactions are probably not expressed in the field and hence would not be submitted to strong selection. In other words, they cannot be counter-selected, if we consider that the cost of their cognitive maintenance is insignificant. Such evolution of solitariness would fit the notion of phylogenetic inertia (phylogenetic constraint *sensu* McKittrick, 1993, or stasis *sensu* Schwenk, 1995) because solitariness did not come with numerous or outstanding behavioural changes. Even though solitariness in Amazonian forest litter can be seen as advantageous to face predation pressures for instance (e.g. from army ants; Grandcolas & Deleporte, 1994; Grandcolas, 1998), the potential of *T. akinetum* for social activity is mainly characterized by nonfunctional plesiomorphic character states and thus cannot be considered as an adaptation (Coddington, 1988; Deleporte, 2002; Grandcolas & D'Haese, 2003).

For subsociality, *P. boleiriana* is behaviourally the most autapomorphic of all species studied. The occurrence of such a high number of autapomorphies fits a model of evolution where a change in social system

**Table 8.** Behavioural differences despite similar habitats

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The most relevant autapomorphic behavioural transitions coded for *Lanxoblatta emarginata* and *Phortioeca nimbata*: two species living under loose bark

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Present ( <i>L. emarginata</i> )	MT (moving toward)/SP (stilt posture) SP (stilt posture)/AC (antennal contact) SA (stilt + antennation)/CB (climbing) SA (stilt + antennation)/GA (go down and hide antennae) SD (stilt then go down)/RO (rotation) RO (rotation)/SP (stilt posture) WP (light withdrawal)/MA (mutual antennation) WP (light withdrawal)/SA (stilt + antennation) WP (light withdrawal)/GD (go down)
Present ( <i>P. nimbata</i> )	SP (stilt posture)/CB (climbing) SA (stilt + antennation)/MT (moving toward) GD (go down)/MT (moving toward) GA (go down and hide antennae)/SP (stilt posture) WD (withdrawal)/GD (go down) GL (leg grooming)/RO (rotation)

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The most relevant autapomorphic behavioural transitions coded for *Eublaberus distanti*: a species living in treeholes

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Present	AC (antennal contact)/PS (pronotal attack) AC (antennal contact)/SJ (jump) MA (mutual antennation)/PS (pronotal attack) MA (mutual antennation)/SJ (jump) CB (climbing)/PP (push with pronotum) SJ (jump)/GD (go down) PP (push with pronotum)/PP (push with pronotum) PS (pronotal attack)/WP (light withdrawal)
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comes with numerous changes in social interactions. These behavioural changes could also result from using a very peculiar habitat (galleries in dead wood) because it is the only xylophagous and wood-living species behaviourally studied so far in this group. In this ‘habitat hypothesis’, the numerous autapomorphies observed in this species could be secondary adaptations (*sensu* Gould & Vrba, 1982), such as ‘tilt’ and ‘stilt’ postures (Legendre *et al.*, 2008b). These postures probably reduce the possibility of being passed by a conspecific in a gallery, which could be advantageous for food or shelter access. Behavioural studies on other xylophagous species or on species living in dead wood (e.g. *Panclhora* spp., *Cryptocercus* spp., Panesthiinae) could help to assess the relative impact of this habitat on sociality and behaviour evolution (Park, Grandcolas & Choe, 2002; Bell, Roth & Nalepa, 2007). Eventually, they would allow refinement of the role of the different evolutionary forces acting on the evolution of cockroach sociality, as was the case in other organisms (Clutton-Brock, 2009; Foley & Gamble, 2009; Hatchwell, 2009; Keller, 2009).

For both *T. akinetum* and *P. boleiriana*, our results would obviously gain from supplementary replicates (closely-related species with the same habitat and social system) to allow any generalization, although such species are unknown. This lack of replication is a recurrent limitation of any homology approach (*sensu* Winkler, 2000), which nonetheless offers strong opportunities in evolutionary biology (Weber & Agrawal, 2012).

#### ECOLOGICAL PRESSURES AND SOCIAL EVOLUTION

Blaberidae, and cockroaches in general, use a diversity of habitats (Bell *et al.*, 2007). Seven different habitats are used by the 13 species investigated in the present study, which limits both intrahabitat comparisons and the establishment of detailed evolutionary scenarios. Indeed, the most ancestral habitats are ambiguous as a result of this high diversity. Nonetheless, two habitat changes can be inferred: toward ‘loose bark’ and toward ‘treeholes’. Our study suggests that these changes do not necessarily imply many behavioural modifications because these habitat

transitions involve no unambiguous and concomitant change in behavioural characters. This result supports the idea that habitat does not play a strong role in Zetoborinae behavioural and social evolution, as already suggested (Grandcolas, 1998).

Intrahabitat comparisons reveal that, even though *L. emarginata* and *P. nimbata* share similar repertoires and behavioural activities (Legendre *et al.*, 2008a), they also have their own behavioural autapomorphies. These results are consistent with the conclusions of van Baaren *et al.* (2002), who qualified *P. nimbata* as a species ‘more gregarious’ than *L. emarginata*. Indeed, we find that *P. nimbata* autapomorphies include proportionally more behavioural transitions favouring social interactions (i.e. behavioural answers that do not lead to stopping or limiting interactions) than those of *L. emarginata*. Behavioural differences between species sharing similar habitats are even more pronounced between *E. distanti* and *B. discoidalis*, a result that has also been shown in adult interactions (Gautier & Forasté, 1982). These outcomes call for moving toward the use of detailed behavioural studies in social evolution rather than the early broadly defined social categories (e.g. ‘gregarious’), as already advocated elsewhere (Desutter-Grandcolas & Robillard, 2003; Grandcolas & D’Haese, 2004; Robillard *et al.*, 2006; Grandcolas *et al.*, 2011; Avilés & Harwood, 2012; Doody *et al.*, 2013).

#### BLABERIDAE PHYLOGENETICS

Zetoborinae is a monophyletic Neotropical group of cockroaches belonging to the Blaberidae family, although its phylogenetic position within the family is not clearly established, with some incongruence between several morphological and molecular studies (Grandcolas, 1993b, 1998; Grandcolas & D’Haese, 2001; Maekawa *et al.*, 2003; Inward *et al.*, 2007; Pellens *et al.*, 2007a; Roth *et al.*, 2009; Djernaes *et al.*, 2012). On the basis of various morphological and behavioural characters, McKittrick (1964) defined the Blaberidae for the first time. At that time, the relationships between subfamilies were not yet established cladistically, and she did not use any formal methodology, so that several groups were para- or polyphyletic (Grandcolas, 1996). Particularly, in her Blaberoïd complex comprising Panesthiinae, Zetoborinae, and Blaberinae, the latter subfamily was paraphyletic. In a cladistic analysis based on morphology, Grandcolas (1993b) defined Zetoborinae and Blaberinae subfamilies as two monophyletic sister-taxa. Later, four molecular studies dealt with Blaberidae phylogenetics using very diverse taxon samples of Zetoborinae and Blaberinae (Grandcolas & D’Haese, 2001; Maekawa *et al.*, 2003; Inward *et al.*, 2007; Pellens

*et al.*, 2007a). Although both subfamilies were often closely related, the comparison of the three most recent topologies reveals that they share only two results: monophyly of the Blaberidae family and of the clade (Geoscapheinae + Panesthiinae). This comparison highlights the instability in Blaberidae phylogenetics, especially in inter-subfamilial relations, as well as the need for further phylogenetic analyses of this group.

Thus, Blaberidae relationships were reconstructed in the present study from molecular, morphological, and behavioural characters to obtain a phylogenetic assumption as robust as possible for investigating social evolution in Zetoborinae (Grandcolas *et al.*, 2001). To our knowledge, this is the first molecular study of this cockroach family testing the monophyly of eight out of the eleven subfamilies.

The phylogenetic analysis with all molecular data resulted in a single most parsimonious tree (see Supporting information, Fig. S1), wherein *D. punctata* (Diplopterinae) was nested within (Blaberinae + Zetoborinae) as sister-taxon to the Zetoborinae *T. akinetum*. This result is surprising and makes little sense from both morphological and biogeographical perspectives. Indeed, Blaberinae and Zetoborinae are exclusively Neotropical, whereas *D. punctata* lives in the Indo-Pacific region. Similarly, Blaberinae and Zetoborinae are each characterized by very peculiar genitalia characters (Roth, 1970a, b; Grandcolas, 1991, 1993b) that they do not share with *D. punctata*. A LBA artefact was thus suspected and corroborated, on the one hand, by the topology obtained when *T. akinetum* was removed, with *D. punctata* then found as sister-taxon to the Malagasy Oxyhaloinae (see Supporting information, Fig. S3), and, on the other hand, by the topology obtained in Bayesian inference (see Supporting information, Fig. S2).

The phylogenetic position of *P. pallida* is another surprising result. *Phoetalia pallida* exhibits apparently typical Blaberinae genitalia with a complex series of spine rings (Roth, 1970b) and other subfamilial morphological synapomorphies (Grandcolas, 1993b). Yet, it is constantly associated with Zetoborinae species in our phylogenetic analyses, which would support an outstanding case of convergence in genitalia morphological evolution between *P. pallida* and Blaberinae species.

Despite these two surprising results, our combined analysis supports the monophyly of Blaberidae and of four subfamilies as previously considered in phylogenetic systematics, if *Phoetalia* is considered as a Zetoborinae. In addition, the clade (Geoscapheinae + Panesthiinae) is also well-supported as monophyletic. By contrast, our results disagree with current taxonomy about Epilamprinae and Diplopterinae, with the former subfamily being under-sampled here and

already known to be phylogenetically and taxonomically heterogeneous (Roth, 1971). Inter-subfamilial relationships are less stable and supported by short branches. These short internal branches could either result from a fast diversification or from a lack of appropriate data to document this period of Blaberidae diversification. Similar to previous analyses, our sampling does not allow this question to be answered, thus prompting the need for further investigations.

Nevertheless, our combined approach with molecular, morphological, and behavioural data derived from the successive event-pairing method (Legendre *et al.*, 2008b) allows a thorough investigation of the evolution of social behaviour within Zetoborinae and Blaberinae, and provides the most documented phylogenetic hypothesis for the lineage under study. This calls for 'integrative' approaches in phylogenetics, in the sense that as many relevant characters as possible should be integrated into the phylogenetic matrix (Grandcolas *et al.*, 2001) to obtain a robust topology for testing evolutionary hypotheses. Diversifying sources of phylogenetic characters will presumably increase the chance of obtaining strong phylogenetic support across the whole topology. In this context, the successive event-pairing method represents a suitable way of integrating behavioural data in phylogenetic analyses.

#### CONCLUSIONS

In the present study, we demonstrate the usefulness of the successive event-pairing method in phylogenetic analyses. With this method, behavioural data from nonstereotyped behavioural sequences bring substantial information to the reconstruction of phylogenetic trees and the investigation of the evolution of behaviour. In our case study, shifting from gregariousness to solitariness does not imply numerous or outstanding changes in behavioural repertoires and dyadic behavioural interactions. Conversely, shifting from gregariousness to subsociality comes with numerous behavioural changes. These results are achieved as a result of a homology approach (*sensu* Winkler, 2000) and show that, as recently suggested (Price *et al.*, 2011), phylogenetic approaches in behaviour investigation are useful for deciphering behaviour evolution and discriminating between its different evolutionary modalities. Also, numerous behavioural studies on cockroaches have focused on aggressiveness (Gautier & Forasté, 1982; van Baaren *et al.*, 2007), notably with the aim of studying the dominance hierarchy in diverse social systems. Here, the 13 species observed show a high level of aggressiveness, which suggests that aggressiveness is a plesiomorphic and widespread condition in this clade. Consequently, aggression would not be that decisive in Blaberidae social evolution and future studies might focus on solicit-

ation and tolerance behaviours to investigate the evolution of sociality in this group.

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