Reevolution of sexuality breaks Dollo's law

Katja Domes[†‡], Roy A. Norton[§], Mark Maraun[†], and Stefan Scheu[†]

[†]Institut für Zoologie, Technische Universität Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany; and [§]College of Environmental Science and Forestry, State University of New York, 1 Forestry 16 Drive, Syracuse, NY 13210

Edited by John C. Avise, University of California, Irvine, CA, and approved March 8, 2007 (received for review January 3, 2007)

The dominance of sexual reproduction is still an unresolved enigma in evolutionary biology. Strong advantages of sex have to exist, because only a few parthenogenetic taxa persist over evolutionary timescales. Oribatid mites (Acari) include outstanding exceptions to the rule that parthenogenetically reproducing taxa are of recent origin and doomed to extinction. In addition to the existence of large parthenogenetic clusters in oribatid mites, phylogenetic analyses of this study and model-based reconstruction of ancestral states of reproduction imply that Crotoniidae have reevolved sexuality from parthenogenetic ancestors within one of those clusters. This reversal in reproductive mode is unique in the animal kingdom and violates Dollo's law that complex ancestral states can never be reacquired. The reevolution of sexuality requires that ancestral genes for male production are maintained over evolutionary time. This maintenance likely is true for oribatid mites because spanandric males exist in various species, although mechanisms that enable the storage of genetically ancestral traits are unclear. Our findings present oribatid mites as a unique model system to explore the evolutionary significance of parthenogenetic and sexual reproduction.

oribatid mites | parthenogenesis | spanandric males | automixis | ancient asexuals

The enigma of the evolution of sex comprises two processes, the origin and the maintenance of sex. Theories on the advantages of sex mainly refer to the improvement of the progeny's fitness in sexual populations despite reducing the overall number of offspring (1–2). Nevertheless, one of the enduring mysteries of biology is the prevalence of sexual reproduction in eukaryotes. Because parthenogenetic species do not waste resources in producing males (the now-classic "two-fold" advantage) and do not break up favorable gene combinations, they should rapidly out-compete sexual species in most environments (1–2). Why this is not true has been debated for decades, with so many answers having been proposed (3–5) that a second enigma has emerged: How could a few animal lineages have maintained parthenogenetic reproduction over considerable evolutionary time, avoiding extinction long enough to radiate and form monophyletic clades? The most studied examples of such "ancient asexual scandals" (1) are darwinulid ostracods (6), bdelloid rotifers (7), and several large clusters within oribatid mites (8–11).

Mites exhibit a bewildering array of genetic systems and reproductive modes (12, 13), and parthenogenetic reproduction has evolved numerous times. Parthenogenesis is most common in Oribatida, a widespread and abundant group of soil invertebrates. An estimated 9% of species are parthenogenetic, which is one to two orders of magnitude higher than in other animal groups (8). Most parthenogenetic oribatid mites are clustered in species-rich clades with no known sexual species, making each such clades an independent "asexual scandal" (8, 9, 11, 14).

The pattern of reproductive modes is most varied in Desmonomata, a speciose group with an age of at least 100 million years (11, 15), probably predating the break-up of Pangea (16). Although most families in this group are either entirely parthenogenetic or sexual, there is also one with mixed reproductive modes (Table 1) (17). All parthenogenetic species have a highly female-biased sex ratio, with most populations having >99% females, whereas sexual species comprise at least 30% males (14, 17). Evidence of these patterns comes from culturing and population studies of a wide range of species throughout the world, representing most known genera (14, 17). However, phylogenetic relationships among the sexual and parthenogenetic taxa have been addressed only superficially.

Of the sexual taxa, Crotoniidae are most puzzling (17). The sexuality of these soil- and tree-dwelling mites may simply reflect the ancestral reproductive mode of Desmonomata, but unlike the other taxa, they are not globally distributed; their range is essentially Gondwanan (Table 1). Also, they are morphologically similar to *Camisia*, a widespread and rather derived genus of the parthenogenetic Camisiidae. The Gondwana distribution and the morphological similarity suggest that Crotoniidae may have evolved from within Camisiidae and thereby reevolved sexuality. The regain of sex would contrast Dollo's law, which states that complex characters never reevolve once they are lost (18). If true, the reevolution of sexuality in oribatid mites would be the first such reversal known in the animal kingdom and would add to the mystique of sex as "the queen of problems in evolutionary biology" (2).

We tested the hypothesis that sexuality reevolved in Crotoniidae by investigating its phylogenetic position among a wide range of sexual and parthenogenetic oribatid mites by using a combined data set of partial sequences of the ribosomal 18S region (18S), the heat shock protein 82 gene (*hsp82*), and the elongation factor 1 alpha gene (*ef1*α).

Results

Phylogenetic analyses with neighbor joining (NJ), maximum likelihood (ML), maximum parsimony (MP), and Bayesian algorithms were based on a supermatrix with 2,897 base pairs and 30 taxa. All algorithms gave nearly identical tree topologies, which largely agree with those based on morphological data and earlier molecular studies (Fig. 1) (9, 19, 20). Although Desmonomata as a whole were paraphyletic, all internal taxa except Camisiidae were monophyletic. The sexual genus *Novonothrus* was basal in Nothridae, supported by high bootstrap and posterior probability values. ML and MP analyses of character evolution consistently assigned sexuality as the ancestral state of Nothridae (Fig. 2).

Author contributions: R.A.N. designed research; K.D. performed research; R.A.N. contributed new reagents/analytic tools; K.D. analyzed data; and K.D., R.A.N., M.M., and S.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Abbreviations: ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining.

Data deposition footnote: The sequences reported in this paper have been deposited in the National Center for Biotechnology Information databank, www.ncbi.nlm.nih.gov (accession nos. AF022027, AF022036, AF022040, AY573591, AY632776, AY632825, AY632837, AY632851, AY632861, DQ090773, DQ090776, DQ090777, DQ090779–83, DQ090786, DQ090789, DQ090793–DQ090802, EF081297–EF081339, EF091416–EF091429, EF093763, EF093770, and EF093781).

[‡]To whom correspondence should be addressed. E-mail: katjadomes@gmx.de.

This article contains supporting information online at www.pnas.org/cgi/content/full/0700034104/DC1.

Table 1. Name of sequenced individuals, fragment length, GenBank accession numbers, distribution, and mode of reproduction for all specimens analyzed in this study

Taxa	Fragment length, bp			GenBank accession nos.			Distribution	Reproductive mode	Refs.
	18S	*hsp82*	*ef1α*	18S	*hsp82*	*ef1α*			
Enarthronota									
Hypochthoniidae									
Hypochthonius rufulus (Koch, 1835)	1,782	531	543	EF091427	DQ090776	AY632861	Holarctic, Seychelles	Parthenogenetic	51, 52, u.o.
Eniochthoniidae									
Eniochthonius minutissimus (Berlese, 1903)	1,759	535	543	EF091428	DQ090773	EF081329	Cosmopolitan	Parthenogenetic	51, u.o.
Lohmanniidae									
Lohmannia banksi (Norton *et al.*, 1978)	1,794	513	543	AF022036	DQ090777	EF081330	U.S.A.	Parthenogenetic	u.o.
Mixonomata									
Nehypochthoniidae									
Nehypochthonius porosus (Norton and Metz, 1980)	1,741	535	543	EF081308	DQ090779	EF081328	U.S.A., Hawaii	Parthenogenetic	53
Phthiracaridae									
Steganacarus magnus (Nicolet, 1855)	1,733	513	543	AF022040	DQ090781	AY632837	Holarctic, U.S.A.	Sexual	51
Atropacarus striculus (Koch, 1835)	1,742	522	543	EF091416	DQ090782	EF081309	Holarctic, Oriental, Australian	Parthenogenetic	32
Euphthiracaroidea									
Rhysotritia duplicata (Grandjean, 1953)	1,741	513	543	EF091417	DQ090780	EF081310	Palearctic	Parthenogenetic	54, u.o.
Desmonomata									
Camisiidae									
Heminothrus paolianus (Berlese, 1913)	1,741	528	543	EF091423	DQ090794	EF081316	Holarctic	Parthenogenetic	17, 51, 55
Platynothrus peltifer (Koch, 1839)	1,741	525	543	EF091422	DQ090793	AY632851	Holarctic, Oriental, New Zealand, Neotropic	Parthenogenetic	17, 40, 51, 56
Camisia biurus (Koch, 1839)	1,741	522	543	EF081302	EF081331	EF081312	Holarctic	Parthenogenetic	17
Camisia spinifer (Koch, 1835)	1,741	522	543	EF091420	EF081332	EF081313	Holarctic, Oriental, South America	Parthenogenetic	17, 51
Crotoniidae									
Crotonia brachyrostrum (Hammer, 1966)	1,741	522	543	EF081303	DQ090796	EF081314	Gondwanan	Sexual	17
Crotonia cf *caudalis* (Hammer, 1966)	1,741	519	543	EF081304	DQ090795	EF081315	Gondwanan	Sexual	17
Hermanniidae									
Hermannia gibba (Koch, 1839)	1,739	510	543	EF091426	DQ090800	EF081327	Holarctic, Seychelles	Sexual	17, 51
Nanhermanniidae									
Nanhermannia coronata (Berlese, 1913)	1,741	535	543	EF091421	DQ090799	AY632825	Holarctic, Neotropic	Parthenogenetic	17, 51, u.o.

By contrast, the sexual genus *Crotonia* clustered within Camisiidae, a large parthenogenetic family of ≈80 species, with *Camisia* being its sister-taxon. In this topology, four successive outgroups of *Crotonia* (two inside and two outside Camisiidae) are entirely parthenogenetic. Monophyly of Camisiidae/Crotoniidae was supported by high bootstrap and posterior probability values (Fig. 1). ML and MP analyses of character evolution assigned parthenogenesis as the ancestral reproductive mode of the Camisiidae/Crotoniidae clade (Fig. 2). ML analysis estimated the rates of loss and regaining of sex to be 0.12 under a symmetrical model of character evolution; under the asymmetrical model, the rate of loss was three times that of regaining sex (0.18 and 0.06, respectively). More biased assumptions for the loss of sex (5:1, 10:1) gave similar results (data not shown).

Results from phylogenetic analyses and the reconstruction of the ancestral states of reproduction support the hypothesis that Crotoniidae reevolved sexual reproduction from parthenogenetic ancestors and contradicts Dollo's law. Therefore, the loss of the complex process of sexuality likely is not irreversible in evolution.

Discussion

The atavistic resurrection of complex ancestral traits, contrary to Dollo's law, appears to be more frequent than commonly thought (21–28). Morphological examples include the reevolution of shell coiling in Gastropoda after 10 million years of absence (22, 23), the reappearance of wings in several lineages of stick insects (24), and the regaining of ancestral muscles in bowerbirds (25). Life history examples include the reevolution of feeding larvae within a group of direct-developing species in the gastropod *Crepipatella* (26) and reversal to a free-living state in several parasites (27). Atavisms are also present in humans (28). Another example relates to reproductive biology; the plant *Hieracium pilosella* (29) reevolved sexuality but from a recent and narrow parthenogenetic lineage. The reevolution of sexuality in ancient parthenogenetic clusters of oribatid mites as suggested by this study is, to our knowledge, previously unrecognized in the animal kingdom.

Much of what has been written about large parthenogenetic clusters in oribatid mites has focused on Desmonomata (9, 10, 14), especially Camisiidae, Malaconothridae, and Trhypochthoniidae. Our data support monophyly of species-rich parthenogenetic taxa within Desmonomata and therefore that parthenogenetic lineages of oribatid mites are not evolutionary "dead-ends"; they have persisted and radiated to form clusters, e.g., the parthenogenetic genus *Nothrus* with 67 species. These lineages of

Table 1. (continued)

Taxa	Fragment length, bp			GenBank accession nos.			Distribution	Reproductive mode	Refs.
	18S	*hsp82*	*ef1α*	18S	*hsp82*	*ef1α*			
Malaconothridae									
Malaconothrus gracilis (van der Hammen, 1952)	1,741	528	543	EF091424	EF081339	EF081311	Holarctic, Neotropic	Parthenogenetic	17, 51
Nothridae									
Nothrus silvestris (Nicolet, 1855)	1,741	535	543	EF091425	DQ090802	AY573591	Holarctic, Australian	Parthenogenetic	17, 51, u.o.
Nothrus silvestris bistilus (Jacot, 1937)	1,741	535	543	EF081305	EF081333	EF081323	East of U.S.A.	Parthenogenetic	17
Nothrus truncatus (Banks, 1895)	1,742	535	543	EF081306	EF081334	EF081322	U.S.A.	Parthenogenetic	17
Novonothrus flagellatus (Hammer, 1966)	1,741	531	543	EF081307	DQ090801	EF081324	New Zealand, Australia, Gondwanan	Sexual	17
Trhypochthoniidae									
Archegozetes longisetosus (Aoki, 1965)	1,748	510	543	AF022027	DQ090798	EF081321	Pantropical	Parthenogenetic	17, 51, 55, u.o.
Mainothrus badius (Berlese, 1905)	1,741	519	543	EF081301	EF081338	EF081318	Holarctic, Neotropic	Parthenogenetic	17
Mucronothrus nasalis (Willmann, 1929)	1,741	528	543	EF081299	DQ090797	EF081319	Boreal, Australian, Neotropic	Parthenogenetic	17, 55, 57
Trhypochthonius americanus (Ewing, 1908)	1,741	528	543	EF081298	EF081337	EF081317	U.S.A.	Parthenogenetic	17, 51, 55
Trhypochthoniellus crassus (Berlese, 1904)	1,741	525	543	EF081300	EF081336	EF081320	Holarctic, Australian, Neotropic, Ethiopic	Parthenogenetic	17, 55
Brachypylina									
Achipteriidae									
Achipteria coleoptrata (Linnaeus, 1758)	1,741	510	543	EF091418	EF081335	AY632776	Holarctic, Neotropic, Oriental	Sexual	u.o.
Carabodidae									
*Carabodes femoralis** (Nicolet, 1855)	1,740	510	543	EF091429	DQ090786	EF081325	Holarctic, Pantropic	Sexual	u.o.
Eutegaeidae									
Eutegaeus curviseta (Hammer, 1966)	1,741	510	543	EF081297	DQ090789	EF081326	Gondwanan	Sexual	u.o.
Phenopelopidae									
Eupelops plicatus (Koch, 1835)	1,740	522	543	EF091419	DQ090783	AY632797	Holarctic, Nearctic	Sexual	51, u.o.
Tectocepheidae									
Tectocepheus velatus (Michael, 1880)	1,746	516	543	EF093781	EF093770	EF093763	Cosmopolitan	Parthenogenetic	51, u.o.

Distribution data are inferred from refs. 16 and 43. u.o., our unpublished observations.
**Carabodes subarcticus* for 18S.

oribatid mites join bdelloid rotifers and darwinulid ostracods (6, 7, 30) as ''ancient asexual'' groups, which challenge the view that sexual reproduction is indispensable for long-term survival and radiation of lineages.

Adding to the ''scandal'' of ancient asexuals (1), the results of our study suggest that Crotoniidae have reevolved sex from parthenogenetic ancestors. Reevolution of sex likely resulted from changes in evolutionary forces. In contrast to Camisiidae which typically colonize soil organic layers, Crotoniidae species frequently colonize trees; soil collections may prove to be accidental for many species (31). Generally, parthenogenesis predominates in oribatid mite communities in soil, whereas the bark of trees and mosses are colonized almost exclusively by sexual species (32, 33). This dominance of sexuality suggests that sexual reproduction is necessary for coping with the more heterogeneous environment (tangled bank hypothesis; see ref. 34) or increased exposure to antagonists (red queen hypothesis; see ref. 35) in aboveground habitats. Furthermore, the reproductive mode is affected by the availability of resources. In soil, the permanent availability of resources (litter material and detritus) may explain the widespread occurrence of parthenogenesis (36). Although *Crotonia* has changed to a tree-dwelling life cycle, the mode of reproduction may have changed accordingly.

How reversion from parthenogenetic to sexual reproduction occurred remains unclear. In animal taxa with cyclical parthenogenesis (intermittent mixis), pure bisexual reproduction can reevolve by the abandonment of the parthenogenetic part of the life cycle, but cyclical parthenogenesis is unknown in oribatid mites (14). Reversion to sexual reproduction may be facilitated because transitions between different modes of reproduction exist in higher mite taxa (12). The studied parthenogenetic oribatid mite species reproduce by automictic thelytoky in which the meiotic maturation division is followed by fusion of haploid nuclei to restore diploidy (37). In automictic species, the reversion to sexual reproduction requires that the ability to produce males has been maintained during long evolutionary periods of parthenogenesis. Many parthenogenetic species of oribatid mites are known to produce rare nonfunctional (spanandric) males (17), as is common for parthenogenetic animals in general (38), including the ''ancient asexual'' darwinulid ostracods (39). In parthenogenetic oribatids, nonfunctionality of males is caused by incomplete spermatogenesis, and females ignore spermatophores if they are formed (40). Nonfunctionality has also been indicated by population genetic studies because populations are unaffected by male presence (41). Why spanandric males persist despite the costs to produce them is unclear. Presumably, such

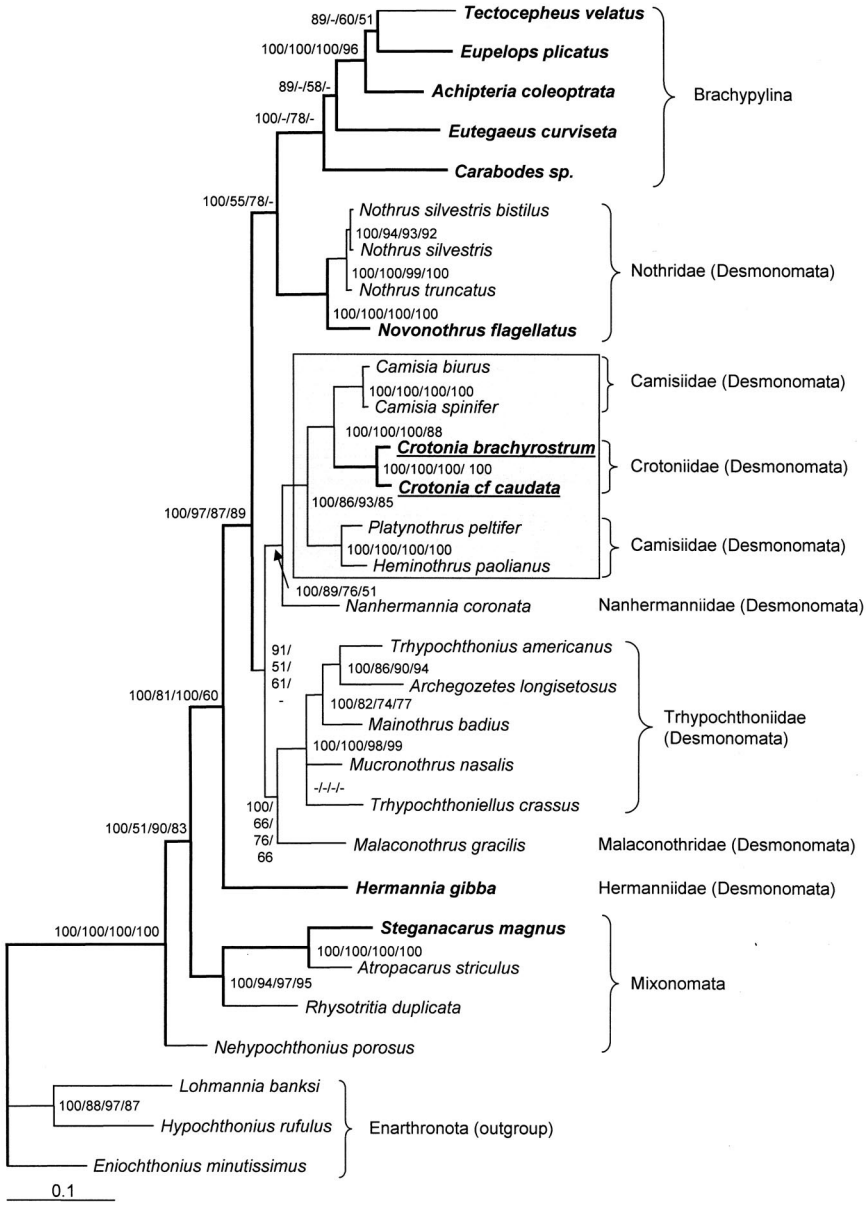

Fig. 1. Bayesian tree of combined sequences of the ribosomal 18S region, the heat-shock protein 82, and the elongation factor 1 alpha of 30 oribatid mite taxa. Enarthronota are used as outgroup. Numbers at nodes represent posterior probabilities for Bayesian analyses and bootstrap support values for NJ, ML, and MP analyses. Sexual lineages are indicated by boldfaced lines and font; species that likely reevolved sexual reproduction are both boldfaced and underlined.

males form as developmental "accidents," as in other parthenogenetic species (42) and, being rare events, the costs of nonfunctional males may be negligible. Irrespective of the mechanisms involved, the occasional formation of spanandric males in parthenogenetic Camisiidae presumably facilitated the capture of functionality of ancestral genes for male production over long evolutionary timescales and therefore the reevolution of sex in Crotoniidae. Knowledge of the genetic and epigenetic mechanisms controlling developmental cascades that lead to male production will answer these questions.

Conclusion

In summary, parthenogenetic radiations are infrequent events in evolution, and the previously unrecognized reevolution of sexual reproduction from parthenogenetic ancestors is even more rare. In general, most parthenogenetic taxa have close sexual relatives and contain few species; in Desmonomata, especially in Camisiidae/Crotoniidae, the pattern is reversed with few sexual taxa within a large cluster of parthenogenetic species. Results of the present study suggest that Crotoniidae indeed reevolved sex, which is a spectacular case of breaking Dollo's law, implying that parthenogenesis is not necessarily an evolutionary dead end. The reevolution of sexual reproduction in Crotoniidae within the ancient clade of parthenogenetic Camisiidae suggests that sexual reproduction is indispensable at certain environmental conditions. Oribatids are an ideal model group to explore these conditions and therefore unravel the enigma of the evolution of sexual reproduction and the conditions under which these reproductive modes prevail.

Materials and Methods

Taxon Sampling. In total, 30 oribatid mite species were sampled. Oribatid mites are commonly ascribed to six major groups, Palaeosomata, Enarthronota, Parhyposomata, Mixonomata,

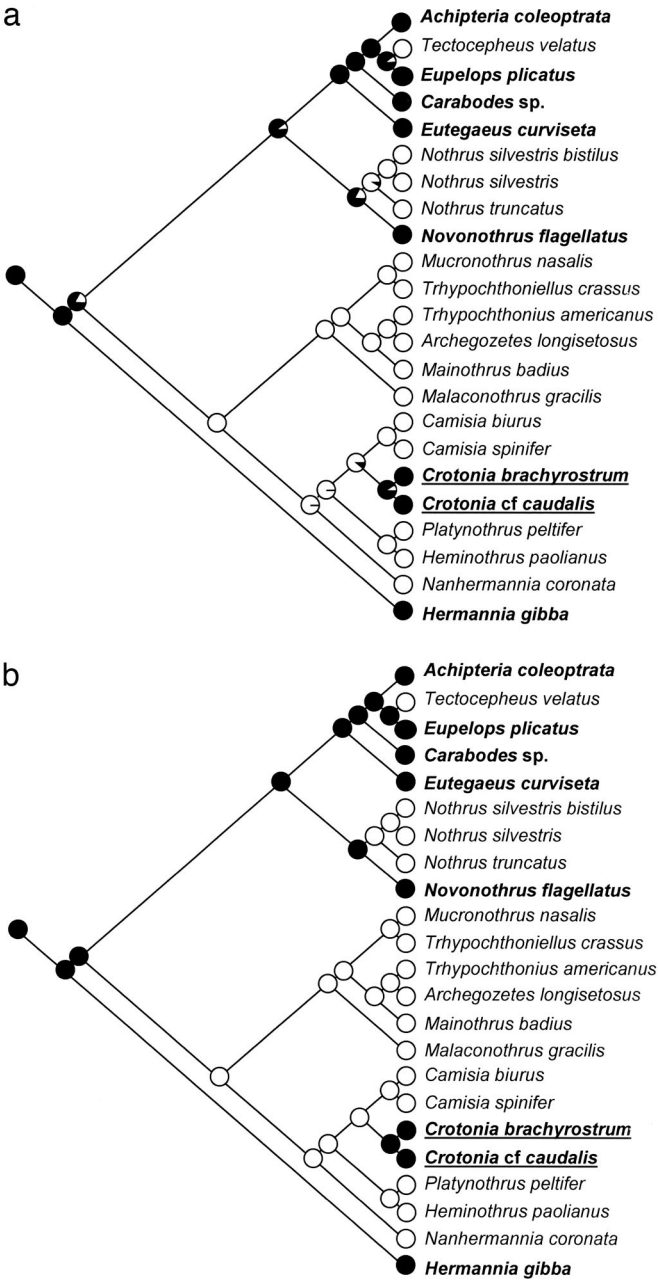

Fig. 2. Cladogram of the Desmonomata on the basis of ML. Ancestral state of nodes is analyzed by ML on the basis of a symmetrical model with equal rates for the loss and regain of sex (*a*) and MP (*b*). Filled circles indicate sexual reproduction; open circles indicate parthenogenetic reproduction. Sexual species are in boldface; species that likely reevolved sexual reproduction are both boldfaced and underlined.

Desmonomata, and Brachypylina (19, 40). Parthenogenetic clusters are most common in Enarthronota and Desmonomata, which are early- and middle-derivative groups, respectively. We focused on Desmonomata, comprising seven families with 36 genera and ≈500 described species (17, 19, 43). In addition to having the large parthenogenetic families Trhypochthoniidae (68 spp.), Malaconothridae (104 spp.), Camisiidae (92 spp.), and Nanhermanniidae (56 spp.), Desmonomata include two families, Crotoniidae (45 spp.) and Hermanniidae (80 spp.), that reproduce only sexually and one family, Nothridae (54 spp.), that has both sexual and parthenogenetic genera. Representatives of all seven families of Desmonomata were included to ascertain whether sexuality in these families appeared to be ancestral or derived with respect to other Desmonomata (Table 1). Camisiidae were most heavily sampled, because a close relationship to Crotoniidae was hypothesized. Other desmonomatan families were represented by a single genus, because their reproductive modes were internally constant.

Several species of Brachypylina, the "higher" oribatid mites, were sampled to ascertain monophyly or paraphyly of Desmonomata. Members of Enarthronota and Mixonomata were sequenced for use as respective outgroups and were selected on the basis of earlier phylogenetic studies (9, 19, 20). Parhyposomata and Palaeosomata were not included, because they are small taxa having no apparent bearing on our objectives.

Oribatid mites were collected from litter and soil at different localities in Germany, Poland, the U.S., New Zealand, and Russia. We complemented the data set with sequences available at GenBank (Table 1).

Sample Preparation, PCR, and Sequencing. Total DNA was extracted from 1 to 10 individuals by using Qiagen (Hilden, Germany) DNeasy kit for animal tissues following the manufacturer's protocol (but elution in 30 μl instead of 400 μl; Qiagen). Amplifications for the18S region, *hsp82*, and *ef1*α were performed either in 50-μl volumes containing 1 μl of each primer (100 pmol/μl), 4–8 μl of DNA, and 25 μl of HotStarTaq Mastermix (2.5 units of HotStarTaq polymerase, and 200 μM each dNTP and 15 mM $MgCl_2$ buffer solution; Qiagen) or in 25-μl volumes by using half the amount of reagents. The primers used and the PCR programs are given in supporting information (SI) Tables 2 and 3. PCR products were visualized on 1% agarose gels and purified by using QIAquick PCR Purification kit (Qiagen). PCR products were either prepared for direct sequencing or cloned by using Qiagen PCR Cloning kit and transformed into *Escherichia coli* Nova Blue Singles competent cells (Novagen, Darmstadt, Germany) by heat shock by using the manufacturer's protocol. The plasmids were purified by using FastPlasmid mini kit (Eppendorf, Hamburg, Germany). DNA was sequenced by Scientific Research and Development (Oberursel, Germany), Qiagen Genomic Services, (Hilden, Germany), or Macrogen (Seoul, Korea). All sequences are available at GenBank (for accession numbers, see Table 1).

Alignment and Phylogenetic Analysis. Because the parameters of the evolutionary models of the three data sets were very similar, DNA sequences of 18S, *hsp82*, and *ef1*α of 30 oribatid mite taxa were combined in a supermatrix and aligned by using the default settings in ClustalX (44); the alignment was modified by eye. The evolutionary model parameters were determined with Modeltest 3.7 (45) by using a hierarchical likelihood ratio test. The model of evolution was TrN+I+G (46) with base frequencies A = 0.3082, C = 0.2238, G = 0.2484, gamma distribution shape parameter α = 0.5819 for four categories of among-site variation, and fraction of invariant sites I = 0.5915. The substitution rates were estimated as A-C, A-T, C-G, and G-T = 1.0, A-G = 2.7550, and C-T = 4.8958. Phylogenetic trees were constructed by using NJ, MP, and ML algorithms as implemented in PAUP* 4b10 (47). MP and ML trees were constructed with a heuristic search of 100 random additions, and the tree-bisection reconnection (TBR) branch-swapping algorithm with the option to collapse zero branch length. A strict consensus tree was constructed for both. Reliability of the branches was ascertained by bootstrap analyses for NJ (100,000 replicates), ML (100 replicates, heuristic search), and MP (10,000 replicates, heuristic search) in PAUP*. Bayesian phylogenetic analysis was performed with MrBayes version 3.1.2 (48) by using the settings for GTR+I+G with three independent runs of 3 million generations and four chains each; rate matrix and base frequencies were

estimated, and trees were sampled every 300 generations. A majority consensus tree was generated by using a burn-in of 2,000.

Ancestral states and the history of character evolution were investigated with parsimony and likelihood algorithms by using the StochChar package in Mesquite (49, 50). Likelihood analyses were calculated under a symmetrical model with equal rates for the loss and regaining of sex and an asymmetrical model with independent rates estimated by ML algorithm. Asymmetrical models with higher rates for the loss of sex (5:1, 10:1) were also tested. Probabilities were calculated assuming equal length for all branches on the basis of the topology of the ML and Bayesian tree.

Separate analyses (NJ, MP, ML, and Bayesian) of the three data sets gave slightly different topologies among desmonomatan families, but internal topologies were identical (data not shown). The Camisiidae/Crotoniidae group was always supported by high support values, and *Novonothrus* occupied a basal position within Nothridae.

We thank J. M. Cianciolo (University of Indiana, Bloomington, IN), N. Lindberg (Uppsala University, Uppsala Sweden), J. Illig (Technische Universität Darmstadt), and D. Sandmann (Technische Universität Darmstadt) for collecting and providing specimens; M. Laumann (Universität Tübingen, Tübingen, Germany) for providing *hsp82* and *ef1α* sequence data; R. H. Cruickshank, M. Heethoff, and I. Schaefer for supporting the data analysis; and U. Brose, D. Mark Welch, W. C. Birky, and two anonymous reviewers for commenting on earlier drafts of the manuscript. This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft).

1. Maynard Smith J (1978) *The Evolution of Sex* (Cambridge Univ Press, Cambridge).
2. Bell G (1982) *The Masterpiece of Nature: The Evolution and Genetics of Sexuality* (Univ of California Press, Los Angeles).
3. Birky CW (1996) *Genetics* 144:427–437.
4. Vrijenhoek RC (1998) in *Advances in Molecular Ecology*, ed Carvalho G (IOS Press, Amsterdam) pp. 151–172.
5. Barraclough TG, Birky CW, Burt A (2003) *Evolution (Lawrence, Kans)* 57:2166–2172.
6. Martens K, Horne DJ, Griffiths HI (1998) in *Sex and Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-Marine Ostracods*, ed Martens K (Backhuys, Leiden, The Netherlands) pp 37–55.
7. Mark Welch D, Meselson M (2000) *Science* 288:1211–1215.
8. Norton RA, Palmer SC (1991) in *The Acari: Reproduction, Development, and Life-History Strategies*, eds Schuster R, Murphy PW (Chapman & Hall, London), pp 107–136.
9. Maraun M, Heethoff M, Schneider K, Scheu S, Weigmann G, Cianciolo J, Thomas RH, Norton RA (2004) *Exp Appl Acarol* 33:183–201.
10. Schaefer I, Domes K, Heethoff M, Schneider K, Schoen I, Norton RA, Scheu S, Maraun M (2006) *J Evol Biol* 18:184–193.
11. Heethoff M, Domes K, Laumann M, Maraun M, Norton RA, Scheu S (2007) *J. Evol. Biol.* 20:392–402.
12. Cruickshank RH, Thomas RH (1999) *Evolution (Lawrence, Kans)* 53:1796–1803.
13. Weeks AR, Marec F, Breeuwer JAJ (2001) *Science* 292:2479–2482.
14. Norton RA, Kethley JB, Johnston DE, O'Connor BM (1993) in *Evolution and Diversity of Sex Ratios*, eds Wrensch DL, Ebbert MA (Chapman & Hall, New York), pp 8–99.
15. Norton RA, Bonamo PM, Grierson JD, Shear WA (1998) *J Paleont* 62:259–269.
16. Hammer M, Wallwork JA (1979) *Biol Skr Dan Vid Selsk* 22:1–31.
17. Palmer SC, Norton RA (1991) *Exp Appl Acarol* 12:67–81.
18. Gould SJ (1970) *J Hist Biol* 3:189–212.
19. Grandjean F (1969) *Acarologia* 11:127–153.
20. Haumann G (1991) *Zur Phylogenie Primitiver Oribatiden (Acari: Oribatida)* (Verlag Technische Universität, Graz, Germany).
21. Marshall CR, Raff EC, Raff RA (1994) *Proc Natl Acad Sci USA* 91:12283–12287.
22. Collin R, Cipriani R (2003) *Proc R Soc Lond B* 270:2551–2555.
23. Pagel M (2004) *Trends Ecol Evol* 19:278–280.
24. Whiting MF, Bradler S, Maxwell T (2003) *Nature* 421:264–267.
25. Rainkov RJ, Borecky SR, Berman SL (1979) *Condor* 81:203–206.
26. Collin R, Chaparro OR, Winkler F, Véliz D (2007) *Biol Bull* 212:83–92.
27. Cruickshank RH, Paterson AM (2006) *Trends Parasit* 22:509–515.
28. Verhulst J (1996) *Acta Biotheoretica* 44:59–73.
29. Chapman H, Houliston GJ, Robson B, Iline IA (2003) *Int J Plant Sci* 164:719–728.
30. Butlin R, Schön I, Griffiths HI (1998) in *Sex and Parthenogenesis: Evolutionary Ecology of Reproductive Modes in Non-Marine Ostracods*, ed Martens K (Backhuys, Leiden, The Netherlands) pp 1–24.
31. Olszanowski Z (1999) *J Nat Hist* 33:233–253.
32. Cianciolo J, Norton RA (2006) *Exp Appl Acarol* 40:1–25.
33. Erdmann G, Floren A, Lisenmair KE, Scheu S, Maraun M (2006) *Pedobiologia* 50:433–441.
34. Ghiselin MT (1974) *The Economy of Nature and the Evolution of Sex* (Univ of California Press, Los Angeles).
35. Van Valen LM (1973) *Evol Theory* 1:1–30.
36. Scheu S, Drossel B (2007) *Proc R Soc Lond B* 274:1225–1231.
37. Wrensch DL, Kethley JB, Norton RA (1994) in *Mites: Ecological and Evolutionary Analysis of Life-History Patterns*, ed Houck MA (Chapman & Hall, New York) pp 282–343.
38. Lynch M (1984) *Q Rev Biol* 59:257–290.
39. Smith JS, Kamiya T, Horne DJ (2006) *Proc R Soc Lond B* 273:1569–1578.
40. Taberly G (1988) *Acarologia* 29:95–107.
41. Palmer SC, Norton RA (1992) *Biochem Syst Ecol* 20:219–231.
42. Groot TVM, Breeuwer JAJ (2006) *Exp Appl Acarol* 39:257–271.
43. Subias LS (2004) *Graellsia* 60(Suppl):1–305.
44. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) *Nucl Acids Res* 24:4876–4882.
45. Posada D, Crandall KA (1998) *Bioinformatics* 14:817–818.
46. Tamura K, Nei M (1993) *Mol Biol Evol* 10:512–526.
47. Swofford DL (1999) *PAUP*: Phylogenetic Analysis Using Parsimony (And Other Methods) Version 4.0* (Sinauer Associates, Sunderland, Massachusetts).
48. Huelsenbeck JP, Ronquist F (2001) *Bioinformatics* 17:754–755.
49. Maddison WP, Maddison DR (2003) *Mesquite: A Modular System for Evolutionary Analysis* (www.mesquiteproject.org.), Version 1.0.
50. Maddison WP, Maddison DR (2005) *StochChar: A Package of Mesquite Modules for Stochastic Models of Character Evolution* (www.mesquiteproject.org), Version 1.06.
51. Grandjean F (1941) *C R Séanc Acad Sci* 212:463–467.
52. Luxton M (1981) *Pedobiologia* 21:312–340.
53. Norton RA, Metz L (1980) *Ann Entomol Soc Amer* 73:54–62.
54. Harding DJL (1969) in *Proceedings of the 2nd International Congress of Acarology*, ed Evans GO (Akademiai Kiadio, Budapest).
55. Palmer SC, Norton RA (1990) *Exp Appl Acarol* 8:445–456.
56. Taberly G (1958) *C R Séanc Acad Sci* 246:1655–1657.
57. Travé J (1973) *Acarologia* 15:522–533.