Biological Journal of the Linnean Society, 2003, **79**, 137–150. With 8 figures

Intraclonal genetic variation: ecological and evolutionary aspects.
Edited by H. D. Loxdale FLS, FRES and G. Lushai FRES

Linkage between sexual and asexual lineages: genome evolution in *Bacillus* stick insects

VALERIO SCALI*, MARCO PASSAMONTI, OMBRETTA MARESCALCHI and BARBARA MANTOVANI

Dipartimento di Biologia Evoluzionistica Sperimentale, Università di Bologna, Italia

The sexually reproducing stick insects *Bacillus rossius* and *B. grandii* are sharply differentiated in terms of allozyme gene alleles; *B. atticus* is a polyclonal automictic parthenogen sister to *B. grandii grandii*. Although well differentiated for coding genes, these hybridize to produce diploid (*B. whitei* = *rossius*/*grandii*) or triploid (*B. lynceorum* = *rossius*/*grandii*/*atticus*) clonal forms which reproduce apomictically. Allozyme analyses of unisexual *Bacillus* clearly establish their relationships from bisexual ancestor species as does the existence in all of them of several clones (especially in *B. atticus*) whose egg maturation allows regular recombination to occur. *Bacillus* taxa share the *Bag320* satellite DNA family within different reproductive frameworks, allowing satellite variant homogenization to be uncoupled from fixation. The nested analysis of monomers reveals different patterns of sequence diversity: sexual reproduction includes both homogenization and variant fixation, whereas the slowing of molecular turnover processes and the absence of syngamy in the parthenogens realizes a similar range of sequence diversity at the level of the individual and supra-individual, but with no fixation. On the other hand, the actual values of sequence diversity appear mostly linked to species traits – range size, copy number of repeats, number of hybrid crosses – and possibly transposon activity, rather than to the reproductive mode. In addition, the mitochondrial genome reveals a comparable level of *cox2* sequence variability in sexual and parthenogenetic taxa, thus adding to clonal variability. From *Bacillus* and other stick insect complexes, an overall picture of genomic diversification of parthenogens is therefore beginning to emerge. To define those animals that reproduce by non-canonical sexual modes (i.e. parthenogenesis, hybridogenesis), but make use of egg and meiotic mechanisms, the term *meta-sexual* is proposed. *Biological Journal of the Linnean Society*, 2003, **79**, 137–150.

ADDITIONAL KEYWORDS: androgenesis – clones – egg cytology – hybridogenesis – mtDNA – parthenogenesis – phylogenesis – polyploids – satDNA – species hybrids.

INTRODUCTION

The predominant way animals reproduce is by sexual reproduction, although several alternative modes also occur. Some of these clearly derive from the sexual mode and animals exploiting non-canonical reproductive mechanisms (i.e. altering some aspects of bisexual reproduction, for example gynogenesis, parthenogenesis and hybridogenesis) are widespread. Suomalainen, Saura & Lokki (1987) provide an extensive list (and analysis) of parthenogenetic invertebrates, whilst Dawley & Bogart (1989) give similar attention to unisexual vertebrates. Being the most specious group, insects are well represented among animals utilizing alternative modes of reproduction. Here we present a summary of what is known on these issues in relation to stick insects.

*Corresponding author. E-mail: scali_v@biblio.cib.unibo.it

STICK INSECTS AND *BACILLUS* TAXA

Phasmatodea is an orthopteroid insect order which includes about three thousand species. Of these, scattered patchily among the different families, around 10% reproduce by facultative (obligate) parthenogenesis, which is generally thelytokous (i.e. producing all-female offspring). Its widespread

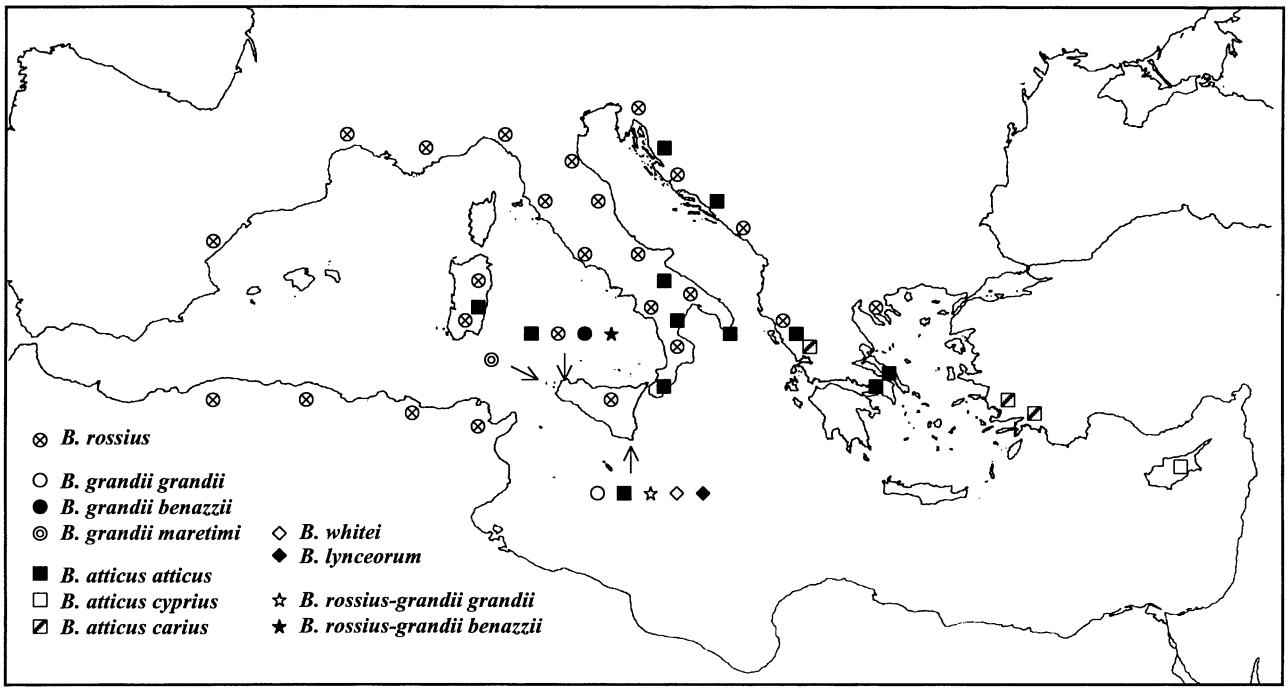

Figure 1. Map of the Mediterranean Basin showing the distribution of *Bacillus* parental (*B. rossius*, *B. grandii* and *B. atticus*) and hybrid taxa. From Mantovani *et al.* (1999).

occurrence has raised questions about the linkage between sexual and asexual lineages, in particular in relation to the egg-cell traits and cytological mechanisms supporting parthenogenetic diffusion, to the persistence (adaptive value) of unisexuals and, as a related but central issue, to their genetic variability. We addressed these questions mainly with respect to the holo-Mediterranean genus *Bacillus* Latreille (Fig. 1).

The genus comprises the following: (1) the bisexual *B. grandii* Nascetti & Bullini ($2n = 33/34$, XO-XX), endemic to tiny areas in Sicily and formally split into three subspecies; (2) the western Mediterranean facultative parthenogen, *B. rossius* (Rossi) $2n = 35/36$, XO-XX, with two Italian races; (3) the eastern Mediterranean *B. atticus* Brunner – at present an all-female obligate parthenogen – forming a complex of three different karyological ($2n = 32$; $2n = 34$; $3n = 48–51$) and allozyme races. These species are the ancestors of the Sicilian thelytokous hybrids: *B. whitei* (= *rossius*/*grandii*) Nascetti & Bullini, and *B. lynceorum* (= *rossius*/*grandii*/*atticus*) Bullini, Nascetti & Bianchi Bullini (Fig. 2). In Sicily, *B. grandii* and *B. rossius* have also hybridized to produce two different hybridogenetic strains (*sensu* Schultz, 1961), which pass on to their progeny an invariant maternal *rossius* haploset, while renewing the paternal genome each generation (hemiclonal reproduction) (Mantovani & Scali, 1992; Mantovani, Passamonti & Scali, 1999). Mitochondrial DNA analysis has shown that unisexual *Bacillus* arose through asymmetrical hybridization events, with only one cross direction of the two possible being realized in nature, *B. rossius* always being the maternal parent. As with most extant hybrid vertebrates (Avise, Quattro & Vrijenhoek, 1992), *Bacillus* hybrids derive from a subset of the matriarchal genealogy of the sexual parent; however, from the available data, it is not possible to assess whether *B. whitei* and *B. lynceorum* both derived from a unique hybridization event (Mantovani *et al.*, 1999; Mantovani, Passamonti & Scali, 2001).

On the whole, *Bacillus* taxa constitute an example of reticulate evolution (i.e. they experience an array of reproductive interactions realizing a complex net of phyletic relationships) and provide a good experimental system to analyse the multifaceted links among sexual and derived clonal taxa.

DISCUSSION

CYTOLOGY OF *BACILLUS* REPRODUCTION

Generally speaking, in the meiocytes of both sexes, the centrosome partially disassembles: in oocytes the centrosome loses the centrioles and its ability to replicate,

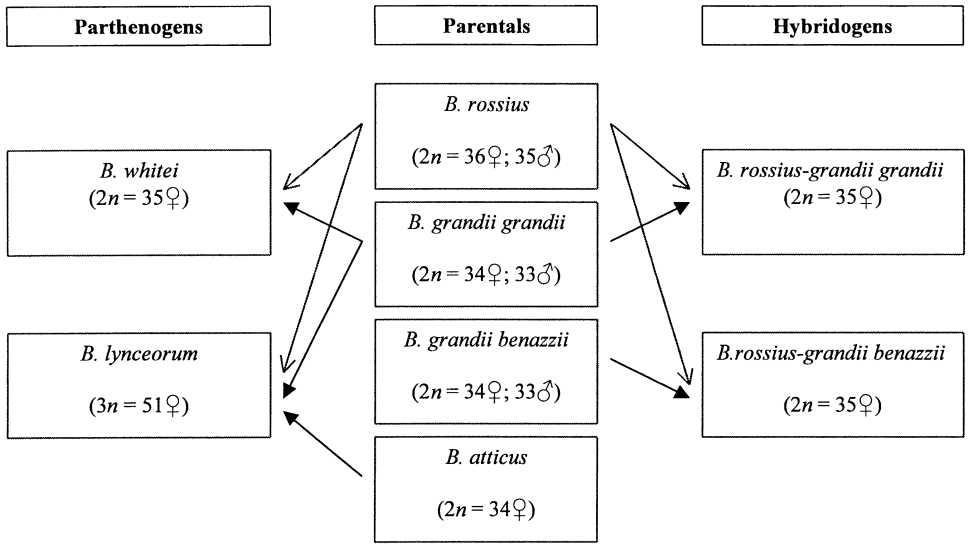

Figure 2. Schematic figure showing the origin of unisexual taxa of *Bacillus*. From Mantovani *et al.* (1999).

but retains the pericentriolar material dispersed throughout the cytoplasm. Conversely, during spermatogenesis, the centrosome is stripped of the pericentriolar material but retains the centriole(s). At fertilization, the two components interact to reassemble a full centrosome (i.e. a micro-tubule organizing centre [MTOC]) around the sperm centriole so that a spermaster is formed; this acquires the ability to replicate and therefore plays a key role in early embryo development by building a functional bipolar spindle (reviewed by Schatten, 1980, 1994; Tassin & Bornens, 1999). However, the relative contribution of each gamete to the zygote centrosome is not invariable, both within sexual and parthenogenetic reproducing species (see Riparbelli *et al.*, 1998; Callaini, Riparbelli & Dallai, 1999; Tram & Sullivan, 2000).

In stick insects, including *Bacillus*, some cell gamete traits linking sexual reproduction to parthenogenesis have been discovered: centrosome dynamics and inheritance in both sexual and parthenogenetic taxa is non-standard when compared with the majority of known animal reproductive systems. In sexually reproducing species of stick insects, the spermatozoon does not appear to contribute the centriole to the fertilized egg; this means that during spermiogenesis, a complete elimination of the male centrosome occurs. In inseminated eggs, no spermaster is formed: an anastral bipolar zygotic spindle is formed from the fusion of a tangle of newly nucleated microtubules (MTs) around both pronuclei (Marescalchi, Zauli & Scali, 2002).

In parthenogenetic eggs, the only source of MTs appears the to be maternal pronucleus, which alone provides a MTOC and the ensuing embryonic anastral spindle; this is then able to organize the embryonic mitoses as efficiently as the spindle of the fertilized eggs. In fact, in *Bacillus,* sexual and parthenogenetic development seems to exploit the ability of chromosome-associated material to nucleate and stabilize spindle MTs (Marescalchi *et al.,* 2002). In summary, the ability of the egg pro-nucleus to fully organize a functional spindle appears to be a major trait accounting for the widespread occurrence of facultative or obligate parthenogenesis in stick insects, since they appear to be preadapted to a disposable sperm contribution to zygote centrosomes. Obviously all parthenogens, including vertebrates, are able to build up their MTOC from egg components alone, but as far as we know, in their sexual relatives, sperm appear to contribute normally to zygote spindle formation (Krioutchkova & Onishchenko, 1999 and references therein; Tram & Sullivan, 2000).

The Mendelian inheritance of genetic variability is based on the standard meiotic mechanism, involving recombination and chromosome assortment: any departure from the standard meiotic features will of necessity have a bearing on genetic structure, at the level of both the individual and population. Generally speaking, the most divergent condition is realized during apomictic parthenogenesis, when meiosis and fertilization are abolished, so that genetically-invariant eggs and progeny are produced. However, several intermediate mechanisms are known in a variety of animals, all of which allow egg development and embryo production through meiotic and/or fertilization alterations; the common by-product of such reproductive modes is a reduction (or very limited level) of genetic variability in the offspring.

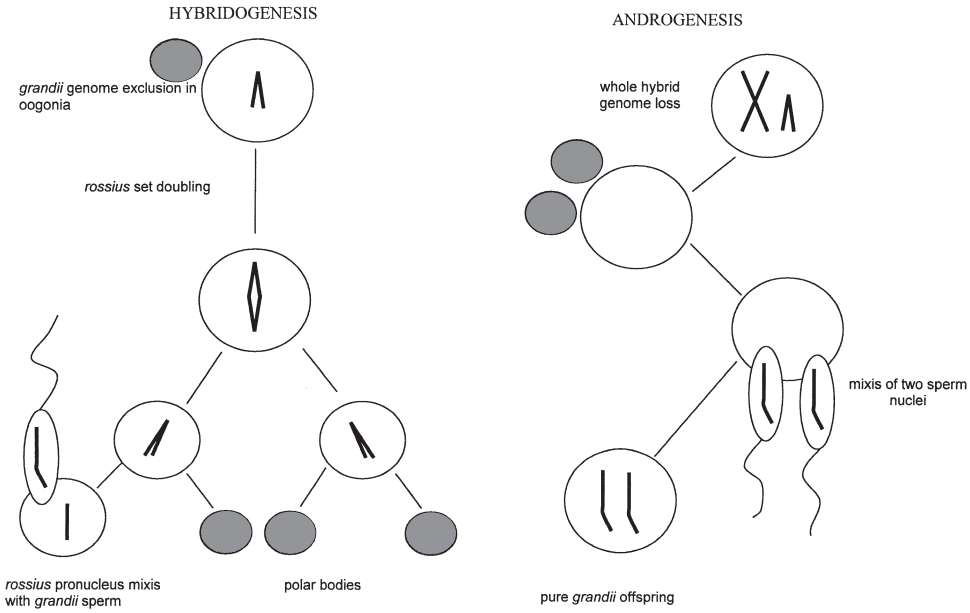

Figure 3. Hybridogenetic and androgenetic cytological mechanisms in *Bacillus*.

The two hybridogenetic strains of *Bacillus* stick insects may be viewed as organisms that produce progeny by skipping the meiotic constraints of heterospecific chromosome pairing, yet paying the price of halving the source of their genetic variability. In other words, the simultaneous elimination of the paternal chromosome haploset (*grandii*) from the hybrid female germ line and the doubling of the maternal one (*rossius*) leads to a chromosomally balanced meiosis in the egg; however, the paired chromosomes are sisters in fact, and no additional genetic variability can be produced by their recombination or assortment. Only the genetic variability derived from the fertilizing spermatozoon of the fathering species contributes each generation to progeny diversification. Hybridogens are therefore hemiclonal in structure (Fig. 3). Moreover, allozyme analysis of the maternal component failed to reveal any variability, so that the *rossius* hemi-clone is always the same in all hybridogenetic populations; this finding is also supported by the genetic homozygosity observed in present day syntopic or geographically close, all-female *B. rossius* populations (Mantovani & Scali, 1990, 1992; Mantovani, Scali & Tinti, 1991; Tinti & Scali, 1993, 1995; Tinti, Mantovani & Scali, 1995). Interestingly, hybridogenetic females can escape hybridity and recover a full genetic diversification of the fathering species in their progeny if their eggs undergo androgenetic reproduction. In the physiologically polyspermic eggs, the maternal *rossius* genome may not perform syngamy with a fathering *grandii* spermatozoon, but degenerate; it may then happen that two male pronuclei fuse instead, thus producing a *B. grandii* progeny of both sexes. These are no longer hemiclonal hybrids but sexually reproducing specimens of *B. grandii* (Fig. 3). Their androgenetic derivation is clear, however, since they possess a heterologous mitochondrial genome of the *rossius* maternal ancestor together with *grandii* nuclear genes (Mantovani & Scali, 1992; Tinti & Scali, 1993, 1995; Mantovani *et al.*, 2001).

The egg maturation of the facultative parthenogen *B. rossius* is realized through a normal meiosis, but its embryonic development is rather unusual: a haploid blastula starts forming and after the production of thousands of cells, diploidization occurs in some of them by means of an anaphase restitution (Fig. 4; zygoid of Suomalainen, Saura & Locki, 1987). This automictic mechanism can be viewed as a gamete multiplication which, after diploidization, generates a female offspring homozygous at all loci in one generation (Pijnacker, 1968; Scali, 1968, 1969). If mated, these females will again produce both sons and daughters. Shifts from sexual to all-female populations must have occurred many times within the different ranges of species (Gasperi, Malacrida & Scali, 1983; Tinti, 1993) and have even been directly witnessed twice (see Scali, 1996). When such a shift occurs, mothers that are heterozygous at some loci will segregate to form different allele combinations in different eggs and thus produce polyclonal, homozygous offspring; if parthenogenetic reproduction is maintained from then onwards, these clones will become fixed genetically at the loci in question (Fig. 4).

In addition, the thelytokous obligate parthenogen *B. atticus* realizes an automictic maturation mechanism of a quite different kind. In diploid females, after

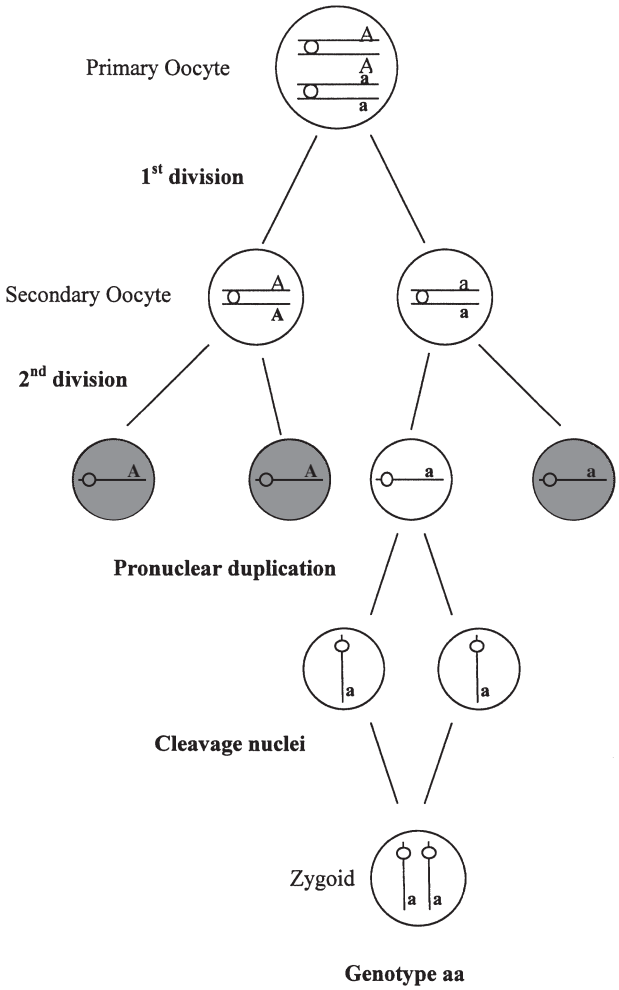

Figure 4. Meiotic and embryonic processes in facultative parthenogenetic *Bacillus rossius*.

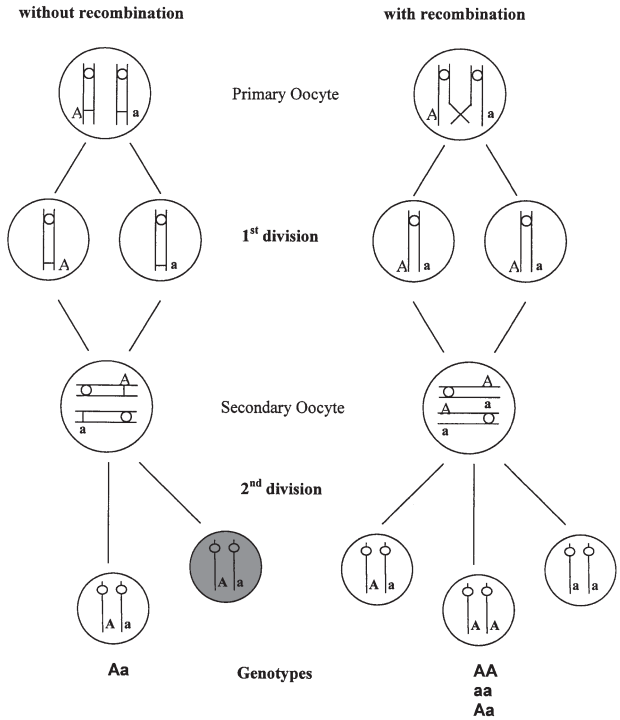

Figure 5. Meiotic and genetic mechanisms in *Bacillus atticus*.

an apparently regular first meiotic division with formation of bivalents and segregation of homologs, both nuclei soon assume a prophasic appearance and fuse to restore a diploid egg nucleus (Fig. 5). This immediately goes through a second division leading to a degenerating polocyte and a quickly dividing, unreduced nucleus from which the parthenogenetic embryo develops (Marescalchi, Pijnacker & Scali, 1993). The maturation mechanism of triploid females keeps the same basic pattern as the diploid ones, the difference being the occurrence of some asynaptic chromosomes which lowers recombination, the formation of heterologous or multivalent chromosome associations, and the unbalanced segregation of chromosomes at anaphase. At any rate, the end result is formation of an unreduced zygoid nucleus (Marescalchi & Scali, in press). This gametogenetic process can account for both the clonal maintenance of chromosomal rearrangements and the transmission of fixed heterozygosities at some loci, but also allows the production of homozygous genotypes from the heterozygous ones during the second division, if an appropriate cross-over has occurred during the first (Fig. 5). This is in full agreement with the complicated pattern of population genetic variability of *B. atticus*, as illustrated below.

B. whitei and *B. lynceorum* share the same apomictic parthenogenetic mechanism characterized by an extra-doubling of DNA, occurring during prophase I. Chromosomes appear to pair briefly and then part again, so that the somatic number is found when the DNA extra-doubling takes place to produce tetrachromatidic chromosomes. Two divisions follow and an unreduced pronucleus plus three polar bodies are produced (Fig. 6). This mechanism follows the meiotic pathway of maturation, but, after the extra-doubling of the somatic number of chromosomes, the process allows the invariant transmission of mostly fixed heterozygosities of the mother to the thelytokous progeny. In both apomicts, at the population level, a few clones can, however, be recognized, so that the species can be described as a collection of a few clones.

VARIABILITY AND PHYLETIC RELATIONSHIPS SUPPORTED BY ALLOZYME AND MITOCHONDRIAL *COX2* GENE ANALYSIS

Allozyme analysis reveals a high genetic variability in *B. grandii* and north African *B. rossius*; Euro-

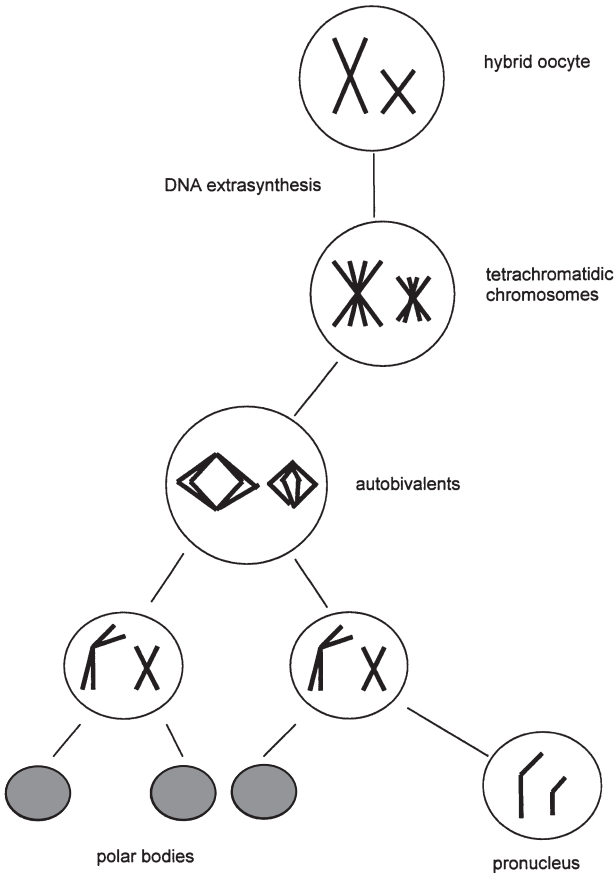

Figure 6. Egg maturation processes in the apomictic *Bacillus whitei* and *B. lynceorum*.

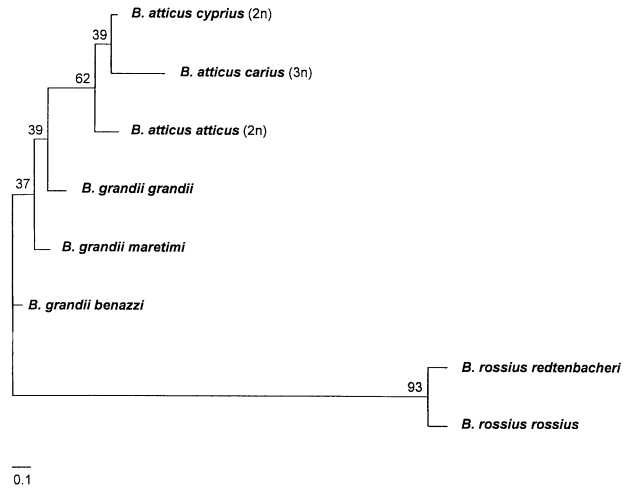

Figure 7. Neighbour-joining tree of genetic relationships among *Bacillus* taxa, based on allozyme analyses. The tree is rooted on *B. rossius* (following the *cox2* analysis) and gives bootstrap values at nodes.

pean populations of the latter have a lower diversification owing to the patchy spreading of facultative parthenogenesis, which brings about fixation of the genetic variability within each specimen, whilst forming different homozygous clones from the segregating maternal heterozygous loci at the population level. The intermingling of bisexual and unisexual populations with their shifting modes of reproduction has certainly contributed to the general pattern of low genetic diversification in European *B. rossius*.

The now obligate parthenogen *B. atticus* also has a rather low genetic variability; however, it shows a complex genetic structure in view of its likely interracial hybrid origin and the occurrence of triploids with a few heterozygous loci, which tend to remain fixed in some populations more than in others.

The species hybrids *B. whitei* and *B. lynceorum* fully reflect the genetic structure of their two and three ancestor species, respectively, with very many fixed heterozygous loci (about 70%) owing to the great differentiation of *B. rossius* on one side and *B. grandii*/*B. atticus* on the other.

Actually, phylogenetic trees among the ancestors of these hybrid, obligate parthenogens, based on Nei's (1978) genetic distance (*D*) values, support such a sharp differentiation (Fig. 7).

In turn, analysis on the mitochondrial *cox2* gene reinforces conclusions shown from allozyme differences and provides evidence that the *B. grandii grandii* subspecies is sister to *B. atticus* and also, that *B. atticus* and *B. grandii* derive from a common ancestral taxon to *B. rossius* (Fig. 8). The matrilinear inheritance of the mitochondrial genome also indicates that both hybridogenetic and parthenogenetic hybrids all have the same maternal ancestor (*B. rossius*) and that hybridization events occurred much later than ancestral splitting (i.e. 1.06 ± 0.53 against 22.79 ± 2.65) (Mantovani *et al.*, 2001).

Clonal structure of non-Mendelian *Bacillus*

Allozyme analysis of the apomictic parthenogen *B. whitei* reveals the presence of six clones from the analysis of 12 populations at 14 enzyme loci (Table 1). The most frequent clone (clone 1) exactly corresponds to the sum of extant Sicilian *B. rossius* and *B. grandii grandii* alleles. The others are always found in females syntopic with clone 1 and differ at a single allele (clones 2, 4, 5, 6) or at two alleles (clone 3), either owing to the presence of parentally unknown alleles or to a homozygous condition which excludes the *grandii* or *rossius* allele. A similar analysis on 13 populations of *B. lynceorum* at 15 enzyme loci produced 15 different clones, the most frequent being 5 and 7 (Table 1) (Mantovani Scali & Tinti, 1992). It is of interest to

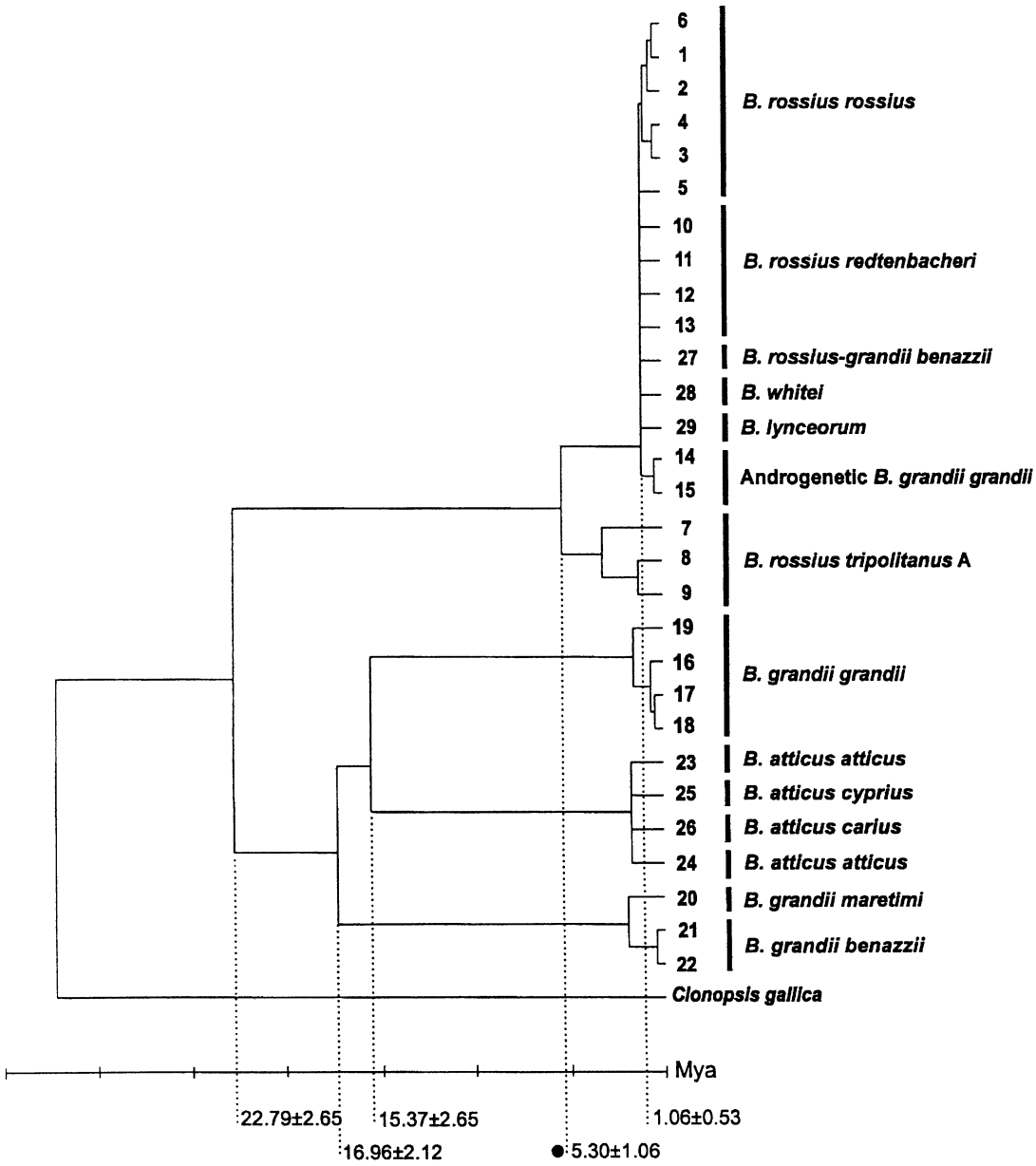

Figure 8. Neighbour-joining linearized tree of the phylogenetic relationships in *Bacillus* taxa. The tree depicts the major cladogenetic events among *Bacillus* taxa and estimates the chronology of divergence using the end of the Messinian (5.3 Myr, black dot) as a calibration for the molecular clock. See Mantovani *et al.* (2001) for details on the statistical methods applied.

note that populations at the core of the hybridization area (Mantovani *et al.*, 1992) are polyclonal (up to seven clones in the Noto area), whereas the peripheral ones are monoclonal. At each locus, *B. lynceorum* clones very often correspond to the sum of extant Sicilian *B. rossius*, *B. grandii grandii* and *B. atticus atticus* alleles, only one clone showing a single new allele at the *Mdh-1* locus. The trihybrid structure of *B. lynceorum* is not always realized though, since at the *G6pdh* locus of clone 14, three *rossius* alleles are found; on the other hand, no *rossius* allele is present at the same locus of clone 3 and at the *Mdh-2* locus of clones 9 and 10. This kind of clonal variation appears to be related to the occurrence of a limited, but also cytologically demonstrable, crossing over event during the early first prophase, just before the DNA extra-doubling stage of both *B. whitei* and *B. lynceorum* (Scali *et al.*, 1995).

Among 158 diploid and 145 triploid *B. atticus* females (24 populations), 52 and 44 clones, respec-

Table 1. Zymotype diffusion and frequency in the apomictic pathenogens *Bacillus whitei* and *B. lynceorum*

	Population	Specimens	Total zymotypes	Specific zymotype/ population*	No. different zymotypes/ population†
B. whitei (2*n*)	12	128	6	1/12 4/2 2/1 3/1 5/1 6/1	4/1 2/3 1/8
B. lynceorum (3*n*)	13	64	15	5/5 7/4 1/3 10/2 2,3/1 4,6/1 8,9/1 11–15/1	5/2 3/1 2/2 1/8

*The first figure refers to the ordinal number of the zymotype, the second to the number of populations in which each zymotype has been found.
†Frequency of different zymotypes per population.

Table 2. Collecting sites, sample sizes and frequencies of zymotypes among diploid (2*n*) and triploid (3*n*) *B. atticus*. Totals refer to the number of specimens and of diploid/ triploid zymotypes. Abbreviations: Cr, Croatian; Gr, Greek; Tr, Turkish; Cy, Cypriot; It, Italian

	Sample size		Zymotypes	
Locality	2*n*♀♀	3*n*♀♀	2*n*	3*n*
Cres (Cr)	9		1	
Mali Losinji (Cr)	9		3	
Zadar (Cr)	9		1	
Dagi Otok (Cr)	4		1	
Igoumenitsa (Gr)		6		3
Parga (Gr)	8	8	4	1
Epidavros (Gr)	10		7	
Paros (Gr)	5		2	
Samos (Gr)		29		10
Iliça (Tr)		7		4
Içemeler (Tr)		26		9
Kushadasy (Tr)		8		4
Bafa Golu (Tr)		18		5
Didim (Tr)		26		4
Bodrum (Tr)	8	17	3	4
Cyprus (Cy)	17		5	
Montegiordano (It)	4		3	
Tindari (It)	8		3	
Milianni (It)	17		8	
Cugni (It)	17		4	
Noto (It)	9		4	
Sardinian demes (It)	22		3	
Totals	156	145	52	44

tively, have been detected upon the analysis of 17 enzyme loci (Mantovani & Scali, 1993; Mantovani, Tinti & Scali, 1995). Since the automictic parthenogenetic mechanism of *B. atticus* allows some recombination and clones may not be strictly maintained as such, the actual allelic combinations are referred to as 'zymotypes'. The number of zymotypes per population ranged from one to ten (highest number: eight in diploids and ten in triploids), apparently without there being a strict relationship to sample size (Table 1). A feature linked to crossover frequency and location is that in both diploid and triploid populations, fixed and non-fixed heterozygosities exist: Epidavros (2*n*) and Samos (3*n*) (both Greek) show no fixed heterozygosity, whereas the Cres, Zadar, Dagy Otok (2*n*) (Croatian), and Parga (3*n*) (Greek) populations show fixation at all heterozygous loci. All other populations show an intermediate condition (Table 2). Furthermore, if for the sake of analysis, these all-female populations are treated as sexual, then it is seen that the Cypriot and Croatian populations among diploids and the Parga (Greek), Samos (Greek), Bodrum (Turkish) populations among triploids show a significant excess of heterozygotes. This complex genetic structuring may be interpreted by taking into account a different frequency of crossing over at some loci owing to their chromosomal location and an impaired synapsis in triploids. A sample of 48 clones (27 diploid and 21 triploid) were analysed in more detail (Table 3): in diploids, two zymotypes are widely represented (number 3 in seven Adriatic samples and number 21 in six Sar-

Table 3. Zymotype diffusion and frequency in the automictic *Bacillus atticus*. In parentheses, samples of 2*n* and 3*n* zymotypes analysed in detail

	Population	Specimens	Total zymotypes	Specific zymotype/ population*	No. different zymotypes/population†
B. atticus (2*n*)	15	156	52 (27)	3/7	8/1
				21/6	7/1
				20/4	5/1
				1/2	4/3
				2/1	3/5
				4–19/1	2/1
				22–27/1	1/3
B. atticus (3*n*)	9	145	44 (21)	11/4	10/1
				18/3	9/1
				10/2	5/1
				13/2	4/4
				17/2	3/1
				1–9/1	1/1
				12/1	
				14–16/1	
				19–21/1	

*The first figure refers to the ordinal number of the zymotype, the second to the number of populations in which each zymotype has been found.
†Frequency of different zymotypes per population.

dinian samples); in triploids, the different zymotypes are more evenly distributed among populations. Furthermore, genotype differences per zymotype reach maximum values of eight and five in diploids and triploids, respectively, whereas the allelic differences amount to four in diploid and as much as 13 in triploid zymotypes.

Distribution and variability of the *Bag320* satellite repeats

Satellite DNAs consist of families of tandemly repeated sequence units, mainly located at the chromosome centromere. They can be isolated through genomic DNA restriction analysis, so that a multimeric ladder is evidenced in ethidium bromide stained gels. Monomers are then cloned and sequenced. SatDNA variability is known to follow a pattern of concerted evolution: this process takes place through the homogenization of new sequence variants within the genome by means of molecular turnover processes (i.e. gene conversion, unequal crossing over, slippage replication, etc.). Variant fixation in the population is then achieved through bisexual reproduction (Dover, 1982, 1986). SatDNA is generally thought to have an ill-defined role and a neutralist viewpoint tends to prevail; however, it is increasingly recognized as being involved in many processes and/or functions such as centromere structure and dynamics, karyotypic evolution and sex/tissue specific transcripts (Tautz, 1993; Elder & Turner, 1995; Renault *et al.*, 1999; Henikoff, Ahmad & Malik, 2001; Schueler *et al.*, 2001; Slamovits *et al.*, 2001).

Bacillus taxa share the same satellite DNA family *Bag320*; therefore they allow analysis of sequence variability in both parental and hybrid taxa. At present, no specific role is attributable to *Bacillus* satDNA; at any rate, the *Bag320* family clearly allows uncoupling of homogenization of satellite variants, which is the result of genomic turnover mechanisms related to molecular features in parthenogens, from the fixation of some sequences linked to sexual reproduction operations (Mantovani *et al.*, 1997). Ongoing research on the *Bag320* satellite repeats (Luchetti *et al.*, in press) is revealing a marked heterogeneity of repeats with different trends and levels in parthenogens and the sexual *B. grandii*. Unisexuals have been characterized electrophoretically (allozymes) as well as mitochondrially (*cox*2 gene), so that *Bag320* variability could be compared among clones: 906 *Bag320* sequences were considered in total and analysed statistically. Owing to their very low copy number, *rossius Bag320* monomers were only obtained from polymerase chain reaction (PCR) amplification products from specimens of *B. rossius*, but were never recovered from its hybrids through cloning; therefore *rossius* monomers were not directly analysed (Luchetti *et al.*, in press).

Table 4. Mean p-distances (pD) ± standard error (SE) of subspecies and species for the bisexual *B. grandii* and the unisexuals *B. atticus, B. whitei* and *B. lynceorum*

Taxon	Mean pD ± SE
B. grandii	0.121 ± 0.008
B. grandii grandii	0.073 ± 0.005
B. grandii benazzii	0.086 ± 0.005
B. grandii maretimi	0.093 ± 0.006
B. atticus	0.146 ± 0.007
B. atticus atticus	0.146 ± 0.007
B. atticus cyprius	0.146 ± 0.007
B. whitei	0.071 ± 0.005
B. lynceorum	0.129 ± 0.008

Concerted evolution in clonal parthenogens versus sexual species

In *B. atticus,* such analyses showed a high overall observed p-distance (pD = 0.146 ± 0.007); no significant differences at any comparison level within the species were found, thereby indicating that the range of variability for sequences of each single female equals that scored among sequences of females of the same population or different populations or different subspecies (Table 4). The same trend of monomer variability was observed in the apomictic hybrids: thus in *B. whitei* and *B. lynceorum*, even considering the clonal structure according to allozyme or mtDNA genotype, statistical analysis of sequences gave non-significant values, both within and among compared samples of the same species.

The pattern of the smeared intraspecific variability evidenced in unisexuals is completely at variance with that found in the three sexual subspecies of *B. grandii*, which show highly significant values of intraspecific differentiation ($P < 0.001$). This, according to Dover's assumptions, can be achieved if both homogenization and fixation are at work to produce a higher homogeneity within the same gene-pool than between different ones, as is clear from subspecific comparisons (Table 4). On the contrary, unisexuals show the same values of variability at all levels, independent of the kind of parthenogenetic mechanism (automictic or apomictic) or unisexual structure (hybrid or non-hybrid). The distinction is even clearer if one considers that *B. grandii grandii*, the sister group of *B. atticus* (Fig. 8; Mantovani *et al.*, 2001), has a pD = 0.073 ± 0.005. Owing to the above-mentioned relationships, it can be readily understood why *B. whitei*, which derived its sequences from *B. grandii grandii*, shows a similarly low value (0.071 ± 0.005); likewise, *B. lynceorum* has a somewhat intermediate value of sequence diversity (0.129 ± 0.008), since this hybrid parthenogen embodies both *B. grandii grandii* and *B. atticus* monomers. When the two kinds of monomers of hybrids were related to the pertinent ancestor species, non-significantly different values were observed (Luchetti *et al.*, in press). It therefore seems that the sequence variability pattern is related mainly to the reproductive mode, whereas its absolute value is rather dependent upon species history (antiquity, possible derivation from a limited subset of ancestor sequences) and range size. As further support for this view, we would like to mention that preliminary data on the *Bag320* satellite family of *B. rossius* – the facultatively parthenogenetic wide-ranging species over the western Mediterranean basin – indicates significantly higher variability than that of *B. grandii*, strictly bisexual but with a very narrow range.

Gene conversion events

The occurrence of a similar array of sequence variability observed in *B. atticus* above the individual level has been explained as being due to the inefficient variant fixation process in the absence of standard fertilization. In addition, the absence of sequence homogenization within individuals could be linked to a slowing effect that the parthenogenetic mode of reproduction induces in molecular turnover mechanisms. Similar considerations could be applied to the variability pattern of sequence observed in *B. whitei*, although this hybrid appears to have had a much shorter time to evolve sequence homogenization. However, in the equally old *B. lynceorum*, in which two monomer types co-occur (i.e. *grandii* and *atticus*), a few sequences show converted DNA stretches (Mantovani, 1998). This is further evidence that in unisexual apomicts too, genome turnover mechanisms, even if slowed down, can operate so that all parthenogenetic *Bacillus* species display a concerted pattern of evolution. The existence of homogenizing mechanisms in parthenogens agrees with data obtained from a different kind of repetitive DNA (ribosomal DNA repeats) of *Daphnia* (Crease & Lynch, 1991) and *Heteronotia* lizards (Hillis *et al.*, 1991; Moritz, 1993). Obligate parthenogenetic *Daphnia* appear to possess, on average, many fewer rDNA repeat types when compared to bisexual and heterogonic strains: from these observations it may be concluded that in clonal organisms, molecular drive should be considerably quicker in homogenizing repetitive DNA sequences. In addition, in parthenogenetic *Heteronotia* lizards of hybrid origin, concerted evolution of rDNA appears to be driven by directional rather than stochastic mechanisms within and among chromosomes: these data support the idea that, even in parthenogens, mutations may spread rapidly through the genome, i.e. through molecular drive. On the whole, homogenizing mechanisms appear to operate in parthenogens, but their rate may differ sharply in different organisms and repetitive DNA families.

CONCLUSIONS

Cytological, allozyme, mitochondrial and satDNA investigations of parental and derived thelytokous *Bacillus* stick insects provide insights into the linkage between these organisms and fully account for the genetic variability of their different genome compartments (mtDNA, satDNA, allozyme genes). First of all, it seems relevant to observe that all-female taxa, either non-hybrid or racial hybrids, retain meiosis or at least allow recombination, as is the case of the facultatively parthenogenetic *B. rossius* or the obligate parthenogen, *B. atticus*. This is a major factor to be considered where genetic variability is concerned. Every source of new gene combinations, such as those provided by the reversal to sexual reproduction for the facultatively parthenogenetic *B. rossius* females, or the cross-over events at heterozygous loci for *B. atticus*, generate genetic variability to produce a wealth of new clones in these unisexuals. Even the apomictic parthenogens, *B. whitei* and *B. lynceorum*, which produce unreduced eggs (Marescalchi & Scali, 2001) do not completely lose meiotic features: not only is the two-stage cascade of meiosis retained, but also a strict clonality is not realized in the offspring, because of a subtle recombinational leakage which appears to occur during the first prophase, particularly in *B. lynceorum,* thus keeping the possibility open for the formation of new clones. Furthermore, the production of triploids, as found in *B. atticus* and *B. lynceorum,* is evidence that incorporation of the entire genome occurs during fertilization of parthenogens. The same seems to apply in the case of an additional stick insect species-complex now under study, the Iberian stick insect genus, *Leptynia* Pantel (Heteronemiidae): both triploid and tetraploid species have been identified (Passamonti, Mantovani & Scali, 1999). This has also repeatedly occurred in several other parthenogenetic/gynogenetic complexes to produce higher rank polyploids (see Dawley & Bogart, 1989; Bullini & Nascetti, 1990; Turgeon & Hebert, 1994; Scali *et al.*, 1995, and references therein). The observation that genome incorporation occurrs in very different animal groups, such as crustaceans, insects and vertebrates, argues in favour of reproductive interactions of unisexuals and related bisexuals and indicates an additional aspect of their links.

With respect to the genomes of unisexuals, satDNA represents a considerable source of variability, although its evolutionary and adaptive significance is still to be completely elucidated. At any rate, satellites are important analytical tools with which to dissect the molecular evolution of the same sequences in related animals with different reproductive modes. Hence, the different trends of variability observed in the bisexual *B. grandii* on the one hand, and the unisexuals on the other, can be related to the different reproductive modes and appear to support Dover's assumption that sexuality acts as a driving force in the fixation of repeated sequences to generate within-population cohesiveness and among-population discontinuity (Dover, 1982, 1986). Yet, if amphimixis is lacking, only turnover mechanisms will operate and no variant fixation can then be achieved. In considering satDNA variability of both sexual and parthenogenetic taxa, purely molecular traits should also be considered. Since copy number has a bearing on homogenization rates (Nijman & Lenstra, 2001), the *B. grandii* pattern of high homogeneity within subspecies could be linked also to the high copy number of *Bag320* monomers (about 15% of the genome); since *B. atticus* has a decidedly lower copy number (2–5% of the genome, Mantovani *et al.*, 1997), its homogenization could be less effective, thus increasing the slowing effect of parthenogenetic reproduction. A similar molecular background could be recognized in *B. whitei* which, due to its derivation and hybrid structure, has inherited a subset of *B. grandii grandii* monomers. In *B. whitei*, the parthenogenetic mode of reproduction plus the hemizygous condition of the *grandii grandii*-like monomers – which actually halves the *grandii* copy number of repeats – could contribute to the homogeneous values of sequence diversity observed at all comparison levels, as it is also found in *B. atticus*.

In *B. lynceorum*, the half-copy numbers of both *atticus* and *grandii* monomers are brought together; notwithstanding its apomictic reproduction and the same duration as *B. whitei*, intrafemale conversion events between the two kinds of sequences have been shown. This dynamic may also reflect a general trend in stabilization of allo-diploid genomes (Hillis *et al.*, 1991; Belyayev *et al.*, 2000). Furthermore in parthenogens, the number and activity of transposable elements and concerted evolution of DNA repeats appear related (Thompson-Stewart, Karpen & Spradling, 1994; Miller *et al.*, 2000; Suellender & Crease, 2001). A lower number and/or activity of transposons in parthenogenetic animals could lead to lower homogenization rates; only targeted analyses will throw some light on the actual role of transposable elements in *Bacillus* taxa, where NOR (nucleolar organizer region) transposition and chromosome breakages have been commonly observed (Manaresi, Marescalchi & Scali, 1991, 1992, 1993; Marescalchi & Scali, 1997). Therefore, stick insect parthenogens are far from being genetically uniform, thus supporting Moritz's ideas on the genetic flexibility of parthenogens (Moritz, 1993). We would also point out that too often, parthenogens are defined as invariant before an accurate analysis of their genetic variability has been performed.

Similarly, the mitochondrial genome appears to add to genetic diversification of unisexual organisms. To

begin with, its high rate of mutation together with the matrilinear inheritance adds to species plasticity, from single specimens to populations. In hemiclonal stick insects, a further way to individual variability is possible when at each generation, the maternal hemi-clone is confronted with new allelic combinations of the fathering male. But the most relevant contribution of the mitochondrial genome to genetic variability occurs in androgens, where *rossius* mitochondria are brought together with *grandii* nuclear genes. It is well known that the co-occurrence of heterospecific mitochondrial and nuclear genomes provides an unusual opportunity for the study of genome evolution. In yeasts, it has been shown that an ongoing transfer of mtDNA fragments to the nucleus occurs at the rate of spontaneous mutation or higher, depending on the fragment size (Thorsness & Weber, 1996). If the same occurs in androgenetic *B. grandii*, this would realize an unexpected feed-back source of genetic variability, a link between unisexuals and the sexual fathering species.

The level of analysis we have performed on stick insects has convinced us of the existence of a graded divergence of thelytokous taxa from the standard sexual condition. A major point is that a lower but real genetic diversification is maintained from hybridogens, through mictic parthenogens, to apomicts, by exploiting several cytological mechanisms. At their core however, it is clear that a meiotic programme, although modified, is maintained during egg maturation: even in apomictic stick insects, a low but effective recombination appears to occur. Furthermore, in parthenogenetic, pseudogamous, hybridogenetic and androgenetic organisms, a germ cell line differentiates to produce and store genetic information for embryonic development in the same way as found in the oocytes of sexually reproducing organisms. In parthenogenetic stick insects, the centrosome inheritance also does not appear to differ from that realized when fertilization occurs. The most obvious case of coincidence of a bisexual and a parthenogenetic egg is that seen in the facultative parthenogenesis of *B. rossius* (and additional stick insect species), where the very same egg can be either fertilized or can develop parthenogenetically in the absence of sperm (Scali, 1968); in addition, the production of degenerating nuclei (polocytes), a constant feature of egg maturation, clearly belong to a meiotic programme. Therefore to consider such all-female organisms as 'asexual' is too inaccurate a description, since it would include them with organisms which reproduce without germ cells. Such a definition would also seem somewhat reductive since it tends to associate sexuality as a whole with meiotic recombination. Therefore, we suggest the term *meta-sexual* for the non-Mendelian reproducing organisms – which however, make use of egg cells and very often still benefit from some recombination – and *meta-sexuality* for their mode of reproduction. These definitions would account for the whole array of the aforementioned reproductive modes derived from sexual animals and would apply to a wide number of animal forms. As a by-product, the new terminology differentiates them from those organisms that make use of only mitotic programming in their reproduction; for these alone, the term *asexual* would seem more appropriate.

REFERENCES

Avise JC, Quattro JM, Vrijenoek RC. 1992. Molecular clones within organismal clones. Mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. *Evolutionary Biology* **26:** 225–246.

Belyayev A, Raskina O, Korol A, Nevo E. 2000. Coevolution of A and B genome in allotetraploid *Triticum dicoccoides*. *Genome* **43:** 1021–1026.

Bullini L, Nascetti G. 1990. Speciation by hybridization in phasmids and other insects. *Canadian Journal of Zoology* **68:** 1747–1760.

Callaini G, Riparbelli MG, Dallai R. 1999. Centrosome inheritance in insects: fertilisation and parthenogenesis. *Biology of the Cell* **91:** 355–356.

Crease TJ, Lynch M. 1991. Ribosomal DNA variation in *Daphnia pulex*. *Molecular Biology and Evolution* **8:** 620–640.

Dawley RM, Bogart JP. 1989. *Evolution and ecology of unisexual vertebrates*. Albany, NY: New York State Museum Press.

Dover GA. 1982. Molecular drive: a cohesive mode of species evolution. *Nature* **299:** 111–117.

Dover GA. 1986. Molecular drive in multigene families: how biological novelties arise, spread and are assimilated. *Trends in Genetics.* **2:** 159–165.

Elder JF, Turner BJ. 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. *Quarterly Review of Biology* **70:** 297–320.

Gasperi G, Malacrida A, Scali V. 1983. Variabilità enzimatica in popolazioni italiane e nord-africane di *Bacillus rossius* (Insecta, Phasmatodea, Bacillidae). *Atti XIII Congresso Nazionale Italiano Di Entomologia*. Sestriere, Torino, Italy, 647–649.

Henikoff S, Ahmad K, Malik HS. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293:** 1098–1102.

Hillis DM, Moritz C, Porter CA, Baker RJ. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. *Science* **251:** 308–310.

Krioutchkova MM, Onishchenko GE. 1999. Structural and functional characteristics of the centrosome in gametogenesis and early embryogenesis of animals. *International Review of Cytology* **185:** 107–156.

Luchetti A, Cesari M, Carrara G, Cavicchi S, Passamonti M, Scali V, Mantovani M. in press. Unisexuality and molecular drive: *Bag320* sequence diversity in *Bacillus* taxa (Insecta: Phasmatodea). *Journal of Molecular Evolution* **56:** in press.

Manaresi S, Marescalchi O, Scali V. 1991. Ag-detected NOR and C-banding patterns in *Bacillus rossius* (Insecta, Phasmatodea) from Sicily. *Caryologia* **44:** 265–286.

Manaresi S, Marescalchi O, Scali V. 1992. The chromosome complement of the hybrid *Bacillus whitei* complex (Insecta, Phasmatodea). II. The repatterned cytotypes. *Cytologia* **57:** 111–119.

Manaresi S, Marescalchi O, Scali V. 1993. The trihybrid genome constitution of *Bacillus lynceorum* (Insecta, Phasmatodea) and its structural-cytogenetic variations. *Genome* **36:** 317–326.

Mantovani B. 1998. Satellite sequence turnover in parthenogenetic systems: the apomictic triploid hybrid *Bacillus lynceorum* (Insecta, Phasmatodea). *Molecular Biology and Evolution* **15:** 1288–1297.

Mantovani B, Passamonti M, Scali V. 1999. Genomic evolution in parental and hybrid taxa of the genus *Bacillus* (Insecta, Phasmatodea). *Italian Journal of Zoology* **66:** 265–272.

Mantovani B, Passamonti M, Scali V. 2001. The mitochondrial cytochrome oxidase II gene in *Bacillus* stick insects: ancestry of hybrids, androgenesis and phylogenetic relationships. *Molecular Phylogenetics and Evolution* **19:** 157–163.

Mantovani B, Scali V. 1990. Preliminary report on a hybridogenetic stick-insect (Phasmatodea): the first case among invertebrates. *Invertebrate Reproduction and Development* **18:** 185–188.

Mantovani B, Scali V. 1992. Hybridogenesis and androgenesis in the stick insect *Bacillus rossius–grandii benazzii* (Insecta, Phasmatodea). *Evolution* **46:** 783–796.

Mantovani B, Scali V. 1993. Genetic structure and phyletic relationships of eastern Mediterranean *Bacillus atticus* Brunner (Insecta, Phasmatodea). *Biochemical Genetics* **31:** 343–362.

Mantovani B, Scali V, Tinti F. 1991. Allozyme analysis and phyletic relationships of two new stick-insects from northwest Sicily: *Bacillus grandii benazzii* and *B. rossius–grandii benazzii* (Insecta, Phasmatodea). *Journal of Evolutionary Biology* **4:** 279–290.

Mantovani M, Scali V, Tinti F. 1992. New morphological and allozymic characterization of *Bacillus whitei* and *B. lynceorum* hybrid complexes (Insecta, Phasmatodea). *Biologisches Zentralblatt* **111:** 87–103.

Mantovani B, Tinti F, Bachmann L, Scali V. 1997. The *Bag*320 satellite DNA family in *Bacillus* stick insects (Phasmatodea): different rates of molecular evolution of highly repetitive DNA in bisexual and parthenogenetic taxa. *Molecular Biology and Evolution* **14:** 1197–1205.

Mantovani B, Tinti F, Scali V. 1995. Genetic structure and similarity relationships of the unisexual Cyprian *Bacillus* (Insecta, Phasmatodea). *Biologisches Zentralblatt* **114:** 299–306.

Marescalchi O, Pijnacker LP, Scali V. 1993. Automictic parthenogenesis and its genetics in *Bacillus atticus* (Insecta, Phasmatodea). *Invertebrate Reproduction and Development* **24:** 7–12.

Marescalchi O, Scali V. 1997. Chromosomal and NOR patterns in the polyclonal stick insect *Bacillus atticus atticus* (Insecta, Phasmatodea). *Genome* **40:** 261–270.

Marescalchi O, Scali V. 2001. New DAPI and FISH findings on egg maturation processes in related hybridogenetic and parthenogenetic *Bacillus* hybrids (Insecta, Phasmatodea). *Molecular Reproduction and Development* **60:** 270–276.

Marescalchi O, Scali V. 2003. Automictic parthenogenesis in the diploid – triploid stick insect *Bacillus atticus* and its flexibility leading to diploid hybrids. *Invertebrate Reproduction and Development*. In press.

Marescalchi O, Zauli C, Scali V. 2002. Centrosome dynamics and inheritance in related sexual and parthenogenetic *Bacillus* (Insecta, Phasmatodea). *Molecular Reproduction and Development* **63:** 89–95.

Miller WJ, Nagel A, Bachmann J, Bachmann L. 2000. Evolutionary dynamics of the *SGM* transposon family in the *Drosophila obscura* species group. *Molecular Biology and Evolution* **17:** 1597–1609.

Moritz C. 1993. The origin and evolution of parthenogenesis in the *Heteronotia binoei* complex: synthesis. *Genetica* **90:** 269–280.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 538–590.

Nijman IJ, Lenstra JA. 2001. Mutation and recombination in cattle satellite DNA: a feedback model for the evolution of satellite repeats. *Journal of Molecular Evolution* **52:** 361–371.

Passamonti M, Mantovani B, Scali V. 1999. Karyotype and allozyme characterisation of the Iberian *Leptynia attenuata* species complex (Insecta Phasmatodea). *Zoological Science* **16:** 675–684.

Pijnacker LP. 1968. Automiktische Parthenogenese in de wandelende Tak *Bacillus rossius* Rossi (Orthoptera, Phasmida). *Genen en Phaenen* **12:** 129–130.

Renault S, Roulex-Bonnin F, Periquet G, Bigot Y. 1999. Satellite DNA transcription in *Diadromus pulchellus* (Hymenoptera). *Insect Biochemistry and Molecular Biology* **29:** 103–111.

Riparbelli MG, Stouthamer R, Dallai R, Callaini G. 1998. Microtubule organization during the early development of the parthenogenetic egg of the Hymenopteran *Muscidifurax uniraptor*. *Developmental Biology* **195:** 89–99.

Scali V. 1968. Biologia riproduttiva del *Bacillus rossius* (Rossi) nei dintorni di Pisa con particolare riferimento all'influenza del fotoperiodo. *Atti della Società Toscana di Scienze Naturali. Memorie* **75:** 139.

Scali V. 1969. Osservazioni citologiche sullo sviluppo embrionale di *Bacillus rossius*. Rendiconti dell'Accademia Nazionale dei Lincei. *Classe Scienze Fisiche Matematiche Naturali, Serie* **VIII:** 486–492.

Scali V. 1996. Distribution, genetic diversification and reproductive mechanisims of unisexual *Bacillus* stick insects. In: *Fifth International Congress of Systematic and Evolutionary Biology.* Budapest: Pars Ltd, Abstract 165.

Scali V, Tinti F, Mantovani B, Marescalchi O. 1995. Mate recognition and gamete cytology features allow hybrid species production and evolution in *Bacillus* stick insects. In: Balletto E, Minelli A, eds. Speciazione: tempo e modi. *Bollettino di Zoologia* **62:** 59–70.

Schatten G. 1980. Motility during fertilization. *International Review of Cytology* **79:** 59–163.

Schatten G. 1994. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Developmental Biology* **165:** 299–335.

Schueler MG, Higgins AW, Rudd MK, Gustashaw K, Willard HF. 2001. Genomic and genetic definition of a functional human centromere. *Science* **294:** 109–115.

Schultz RJ. 1961. Reproductive mechanism of unisexual and bisexual strains of the viviparous fish *Poeciliopsis*. *Evolution* **15:** 302–325.

Slamovits CH, Cook JA, Lessa EP, Rossi MS. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in south American Tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae): a phylogenetic approach. *Molecular Biology and Evolution* **18:** 1708–1719.

Suellender BW, Crease TJ. 2001. The behaviour of a *Daphnia pulex* transposable element in cyclically and obligately parthenogenetic populations. *Journal of Molecular Evolution* **53:** 63–69.

Suomalainen E, Saura A, Lokki J. 1987. *Cytology and evolution in parthenogenesis.* Boca Raton, Florida: CRC Press.

Tassin AM, Bornens M. 1999. Centrosome structure and microtubule nucleation in animal cells. *Biology of the Cell* **91:** 343–354.

Tautz D. 1993. Notes on the definition and nomenclature of tandemly repetitive DNA sequences. *Experientia* **67:** 21–28.

Thompson-Stewart D, Karpen G, Spradling AC. 1994. A transposable element can drive the concerted evolution of tandemly repetitious DNA. *Proceedings of the National Academy of Sciences, USA* **91:** 9042–9046.

Thorsness PE, Weber ER. 1996. Escape and migration of nucleic acids between chloroplast, mitochondria, and the nucleus. *International Review of Cytology* **165:** 207–234.

Tinti F. 1993. Morphological, genetic and chromosomal characterization of Corsican and Spanish *Bacillus rossius* (Insecta, Phasmatodea). *Vie et Milieu* **48:** 109–117.

Tinti F, Mantovani B, Scali V. 1995. Reproductive features of homospecific hybridogenetically-derived stick insects suggest how unisexuals can evolve. *Journal of Evolutionary Biology* **8:** 81–92.

Tinti F, Scali V. 1993. Chromosomal evidence of hemiclonal and all-paternal offspring production in *Bacillus rossius-grandii benazzii* (Insecta, Phasmatodea). *Chromosoma* **102:** 403–414.

Tinti F, Scali V. 1995. Allozymic and cytological evidence for hemiclonal, all-paternal and mosaic offspring of the hybridogenetic stick insect *Bacillus rossius–grandii grandii*. *Journal of Experimental Zoology* **273:** 149–159.

Tram U, Sullivan W. 2000. Reciprocal inheritance of centrosomes in the parthenogenetic Hymenopteran *Nasonia vitripennis*. *Current Biology* **10:** 1413–1419.

Turgeon J, Hebert PDN. 1994. Evolutionary interactions between sexual and unisexual members of *Cyprinotus* (Ostracoda: Cyprididae). *Evolution* **48:** 1855–1865.