Insect Molecular Biology

Insect Molecular Biology (2010), **19** (Suppl. 1), 91–98 doi: 10.1111/j.1365-2583.2009.00915.x

The distribution of microsatellites in the *Nasonia* parasitoid wasp genome

B. A. Pannebakker*†, O. Niehuis‡, A. Hedley*, J. Gadau‡ and D. M. Shuker*§

**Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, UK; †Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, Groningen, the Netherlands; ‡School of Life Sciences, Arizona State University, Tempe, AZ, USA; and §School of Biology, University of St Andrews, St Andrews, UK*

Abstract

Microsatellites are important molecular markers used in numerous genetic contexts. Despite this widespread use, the evolutionary processes governing microsatellite distribution and diversity remain controversial. Here, we present results on the distribution of microsatellites of three species in the parasitic wasp genus *Nasonia* generated by an *in silico* data-mining approach. Our results show that the overall microsatellite density in *Nasonia* is comparable to that of the honey bee, but much higher than in eight non-Hymenopteran arthropods. Across the *Nasonia vitripennis* genome, microsatellite density varied both within and amongst chromosomes. In contrast to other taxa, dinucleotides are the most abundant repeat type in all four species of Hymenoptera studied. Whether the differences between the Hymenoptera and other taxa are of functional significance remains to be determined.

Keywords: evolutionary genomics, microsatellites, comparative analysis, arthropods, *Nasonia*.

Introduction

Short tandem repetitive elements (microsatellites) are one of the most important sets of molecular genetic markers in use today (Ellegren, 2004). Their practical use is common in population genetic and molecular ecology studies, providing data on aspects of population genetic structure, genome-wide levels of genetic diversity, and patterns of paternity and kinship in natural populations (Goldstein & Schlötterer, 1999; Avise, 2004; Selkoe & Toonen, 2006; for case studies with the present species see Burton-Chellew *et al.*, 2008; Grillenberger *et al.* 2008). In addition, microsatellites are useful markers for linkage and association mapping of traits of economic, medical or ecological interest (eg Mackay, 2001; Erickson *et al.*, 2004; Hirschhorn & Daly, 2005; Knott, 2005). Moreover, microsatellite loci have also been implicated in a number of human diseases, including several cancers (Ashley & Warren, 1995; Oda *et al.*, 2005; Pearson *et al.*, 2005), with disease associated with microsatellite expansion or instability.

The utility and medical importance of microsatellites stem from their hypervariablity and their abundance in some genomes (Ellegren, 2004). Despite this, the processes underlying the evolution of microsatellites remain controversial and the origin of this variability an ongoing puzzle. The nature and properties of the mutational process has long been of interest (Goldstein & Schlötterer, 1999; Ellegren, 2004), both for providing a robust basis for population genetic inference and for understanding their contribution to hereditary diseases and somatic mutations. More generally, explaining different patterns of microsatellite diversity across species is of interest if such patterns provide clues about broader processes of genome evolution. Given that some of the suggested uses of microsatellites (for instance as proxies of genome-wide levels of diversity) make certain assumptions about how microsatellite diversity is generated and maintained (which might be wrong, or violated, by the practicalities of developing and using them as markers, e.g. Väli *et al.*, 2008), understanding these rather fundamental aspects of microsatellite biology are clearly important. Fortunately, to some extent the issues concerning the evolution of microsatellite loci can be resolved empirically. The appearance of whole-genome sequences across a diversity of taxa now presents us with the opportunity both to describe basic patterns of microsatellite distribution and diversity across whole genomes and to begin to address fundamental aspects of

Correspondence: B. A. Pannebakker, Evolutionary Genetics, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, NL-9750 AA Haren, the Netherlands. Tel.: + 31 50 63 2128, fax: + 31 50 363 2348, e-mail: B.A.Pannebakker@rug.nl

Table 1. Microsatellite content of arthropod genomes for each of the 12 studied arthropod species

Species	Physical genome size (Mb)	Number of repeats	Microsatellite content (% genome)	Microsatelllites per Mb genome	Average distance microsatellites (bp)
Nasonia vitripennis	332.5	92 203	0.96	277	3 606
Nasonia giraulti	332.5	119 975	1.27	361	2 771
Nasonia longicornis	332.5	93 233	0.91	280	3 566
Apis mellifera	264.1	81 277	0.77	308	3 249
Acyrthosiphon pisum	303.2	72 575	0.53	239	4 178
Anopheles gambiae	264.1	69 181	0.71	262	3 818
Aedes aegypti	804.4	51 664	0.18	64	15 570
Drosophila melanogaster	176	21 446	0.5	122	8 207
Drosophila simulans	146.7	12 363	0.17	84	11 866
Bombyx mori	508.6	29 617	0.13	58	17 173
Ixodes scapularis	2484.1	100 169	0.11	40	24 799
Daphnia pulex	332.5	31 008	0.18	93	10 723

Physical genome sizes were obtained from Gregory (2006), in which *N. longicornis* and *N. giraulti* are assumed to have similar genome sizes to *N. vitripennis*.

their evolution in a comparative setting. Here, we consider the distribution and evolution of microsatellites in the parasitic wasp genus *Nasonia*.

Nasonia is a genus of generalist gregarious wasps that parasitize large dipteran pupae (Whiting, 1967). *Nasonia* has been used extensively for behavioural and genetic research for many decades (Saul & Kayhart, 1956; Whiting, 1967; Beukeboom & Desplan, 2003; Pultz & Leaf, 2003). In the last 10 years it has emerged as a viable genetic model system, in particular for the genetic dissection of complex traits (e.g. Gadau *et al*., 2002; Velthuis *et al*., 2005, Beukeboom *et al*., 2010) and genic incompatibilities (Breeuwer & Werren, 1995; Gadau *et al*., 1999; Ellison *et al*., 2008; Niehuis *et al*., 2008; Gibson *et al*., 2010) and for comparative developmental genetics (e.g. Pultz *et al*., 2005; Lynch *et al*., 2006; Brent *et al*., 2007; Olesnicky & Desplan, 2007). This emergence of *Nasonia* as a next-generation model organism has recently been given further impetus by the sequencing of the genomes of three *Nasonia* species: *Nasonia vitripennis*, *Nasonia girault*i and *Nasonia longicornis* (Werren *et al*., 2010). Although a set of microsatellite markers exists for *Nasonia* (Pietsch *et al*., 2004), detailed linkage mapping and population genetic studies would greatly benefit from having additional microsatellite markers. The current availability of an assembled genome for the *Nasonia* genus allows for an efficient and cost-effective *in silico* approach for the detection of microsatellites (Sharma *et al*., 2007). In addition, such an approach allows for the study of microsatellite evolution and distribution throughout the genome. In this paper, we describe the number and characteristics of the dinucelotide to hexanucleotide microsatellite loci in the three *Nasonia* species, and their position within the genome of *Nasonia vitripennis*. We then compare these findings with results from nine other arthropod species (the insects *Apis melifera*, *Acyrthosiphon pisum*, *Aedes aegypti*, *Anopheles gambiae*, *Drosophila melanogaster*, *Drosphila simulans* and *Bombyx mori*, the arachnid *Ixodes scapularis* and the crustacean *Daphnia pulex*).

Results

Amongst the 12 arthropod genomes surveyed, the *N. vitripennis* genome has the highest microsatellite abundance (approximately 1% of the genome). The genomes of *N. giraulti* and *N. longicornis* show similarly high microsatellite abundance (Table 1, Fig. 1). There are thus between 90 000 and 120 000 microsatellite loci in the *Nasonia* parasitoid wasp genomes. The genome of the honey bee, *Apis mellifera,* also shows a high microsatellite abundance, which suggests this might be a Hymenoptera-specific pattern. Our results for *A. mellifera* confirm earlier reports (Meglécz *et al*., 2007) of high microsatellite abundance in this species compared to other insects. The estimates for local microsatellite abundance in *N. vitripennis* show that microsatellite densities vary by more than an order of magnitude, both within and amongst the five chromosomes (Fig. 2). For example, on chromosome 2 the density of repeats goes from fewer than 10 kb per Mb to more than 30 kb per Mb in fewer than 5 cM. In terms of comparisons amongst chromosomes, chromosome 5 is less densely populated with microsatellites than the others, whereas chromosome 2 has the most loci (Fig. 2).

Based on similarities in their flanking sequence regions, the three *Nasonia* species share approximately 17–23% orthologous microsatellite loci (Fig. 3). Taken at face value, *N. vitripennis* shows the highest percentage of loci orthologous to those found in the congeners (around 60%). In *N. girault*i and *N. longicornis* the number of orthologous microsatellite loci with other species is lower, at 42 and 48%, respectively. These latter two species share the most recent common ancestor and are derived from *N. vitripennis* (Campbell *et al*., 1993). The number of loci shared amongst the species, however, is a function of the

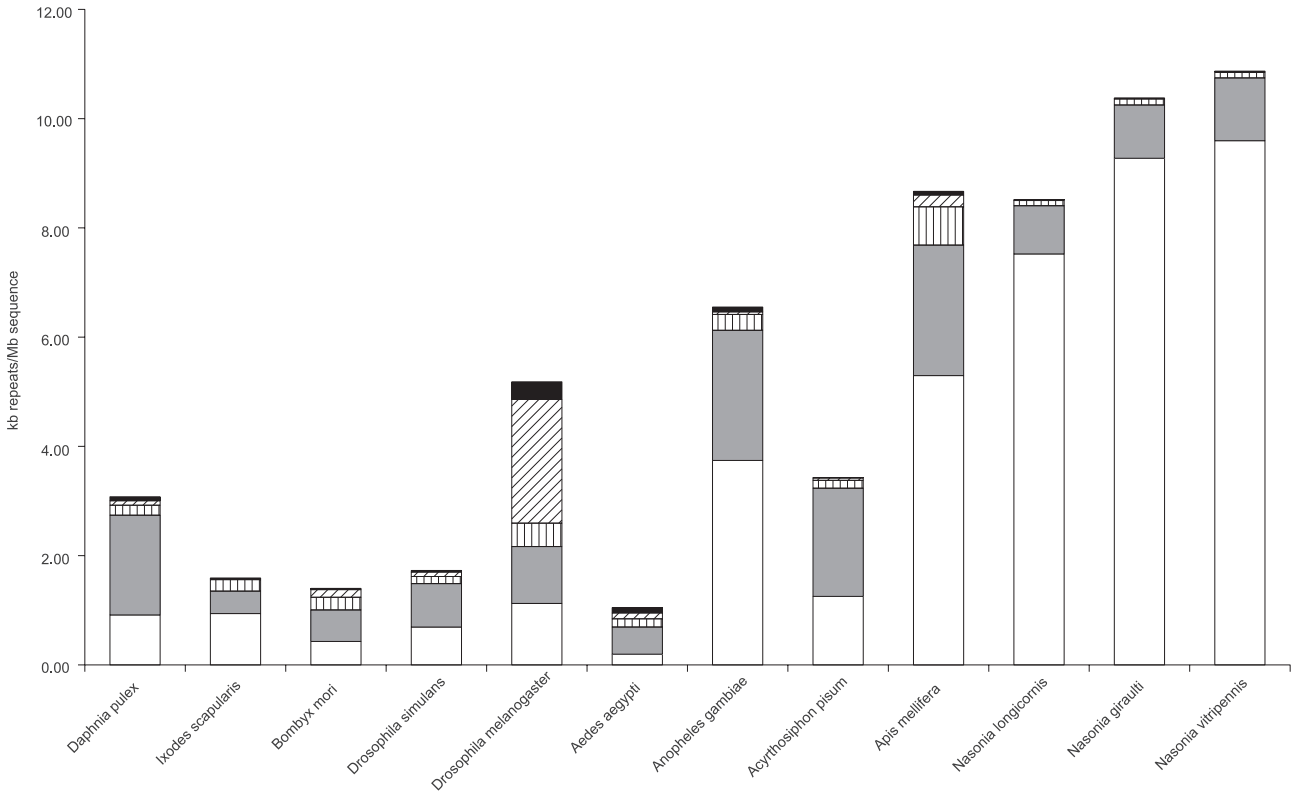

Figure 1. Microsatellite abundance (kb repeat sequence per Mb of analysed DNA) generated by MSATFINDER for the 10 arthropod genomes studied (see Experimental procedures for further details). Data are shown for di- (open area), tri- (grey), tetra- (vertical lines), penta- (diagonal lines) and hexanucleotide repeats (black).

sequence coverage of the genomes analysed, which for these species is a single-fold coverage in *N. girault*i and *N. longicornis*, and a sixfold coverage in *N. vitripennis*.

In terms of microsatellite composition, dinucleotide motifs are the most abundant microsatellites in the three *Nasonia* genomes, making up 89% of all microsatellites. In the genome of *A. mellifera*, dinucleotides are also the most abundant motif, although here they make up only 61% of all microsatellites. Most non-Hymenopteran arthropod species also show an over-representation of a single motif: dinucleotides in *Anopheles gambiae* and *Ixodes scapularis*, trinucleotides in *Aedes aegypti*, *Acyrthosiphon pisum*, *Bombyx mori* and *Daphnia pulex*, and pentanucleotides in *Drosophila melanogaster*. In *Drosophila simulans*, both dinucleotides and trinucleotides are the most abundant motif. Even though dinucleotides are over-represented in the examined Hymenoptera, the abundances of the motif types are different. In *Nasonia*, the most abundant motif type is AG repeats (5.35 Kb repeats/Mb analysed DNA), whereas in *A. mellifera* AT is the most abundant motif type (2.60 kb repeats/Mb analysed DNA) (Fig. 4). Two other species show a high abundance of a single motif type type, in *Anopheles gambiae* AC repeats (2.02 kb repeats/Mb analysed DNA) and in *Acyrthosiphon pisum* AAT repeats are the most abundant motif types (1.53 kb repeats/Mb analysed DNA). In the other species, the different motif types are more evenly represented in the genome (Fig. 4).

Discussion

A comparative genomics approach provides important information to help us better envisage the evolutionary forces shaping microsatellite distribution and diversity. Here, we have shown a great variability in microsatellite abundance in the genomes of the 12 arthropod species examined. Our analysis shows that the three *Nasonia* species and *Apis*, i.e. the Hymenoptera tested, have a higher microsatellite abundance than the other arthropods. In Hymenoptera, the microsatellite distribution is skewed towards dinucleotide repeats, whereas the other arthropod species show a more even distribution across repeat types. To what extent might these features be Hymenoptera-specific, and if so, why?

An important feature of hymenopteran biology is their haplodiploid mode of reproduction: males are haploid and usually develop parthenogenetically from unfertilized eggs, whereas females are diploid, developing from fertilized eggs (Cook, 1993). Haplodiploid reproduction has implications for the genetics of Hymenoptera at many

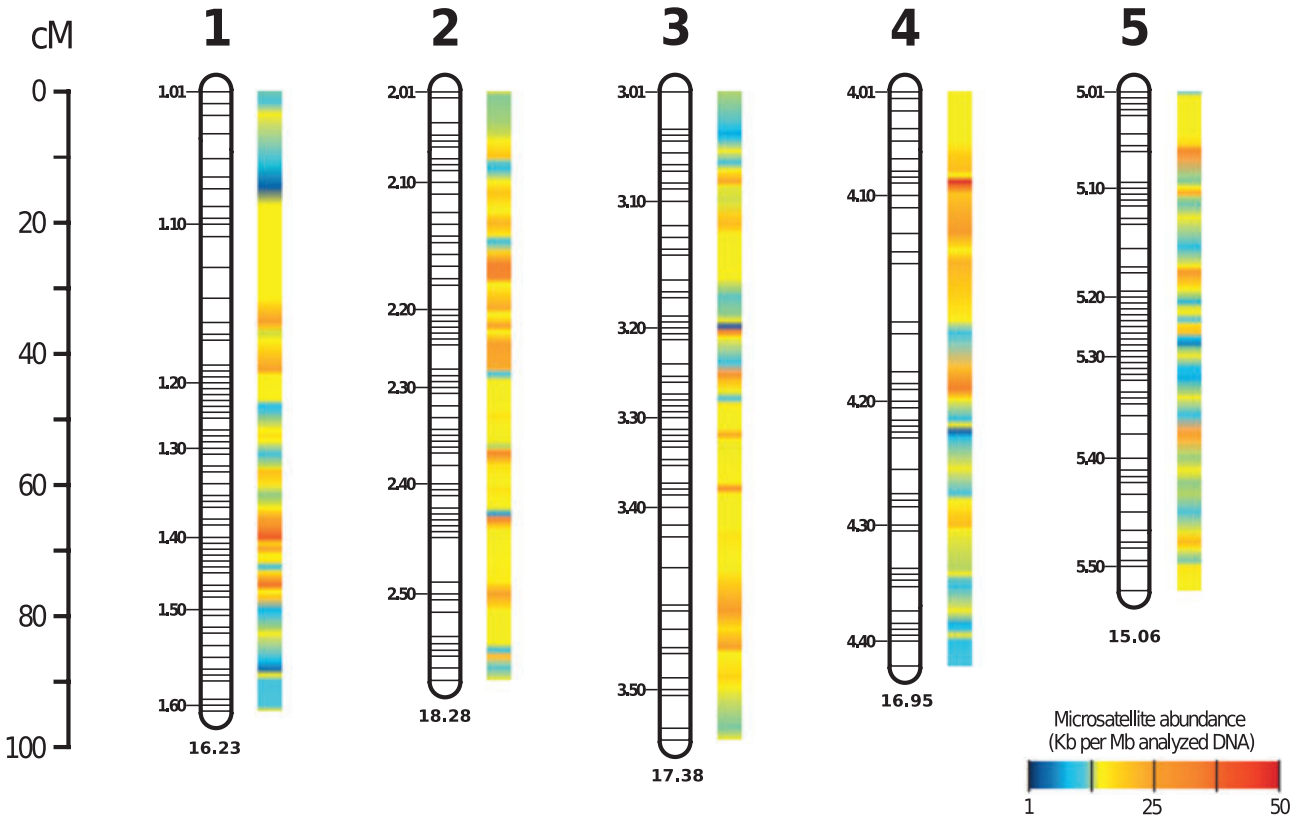

Figure 2. Heat map showing the local microsatellite abundance (Kb repeats per Mb analysed DNA) in regions of the *Nasonia vitripennis* genome, projected on the high-density linkage map of the *Nasonia* genome published by Niehuis *et al.* (2010). Horizontal bars (1.01–5.51) represent clusters of markers with no recombination amongst them (for details see Niehuis *et al.*, 2010). The local microsatellite abundance was calculated for 264 marker clusters with an average size of 1.69 cM (see Supporting Information Table S2). Mean microsatellite abundance is given below each chromosome. The heat map represents 93% of the microsatellites found in the *N. vitripennis* genome.

levels (Crozier, 1977), and could play a role in explaining the observed pattern of microsatellite diversity and abundance. For example, theory suggests that because lethal and deleterious mutations are directly exposed to selection in haploid males, haplodiploid organisms should have a lower genetic load, i.e. less reduction in fitness as a result of deleterious alleles maintained at mutation-selection balance (Crozier, 1985; Werren, 1993). Empirical evidence following meta-analysis supports this prediction (Henter, 2003). A lower genetic load can in turn result in less stringent selection for the maintenance of DNA (mismatch) repair mechanisms, which may play an important role in microsatellite accumulation and evolution (Strand *et al.*, 1993; Ellegren, 2004; Schlötterer, 2004; Buschiazzo & Gemmell, 2006). A recent study by C. Smith (Werren *et al.* 2010), in which the *Nasonia* genome was screened for a wide array of additional types of repetitive DNA, including interspersed repeats and low complexity DNA sequences, confirmed that *Nasonia* has one of the most repeat-rich insect genomes studied so far.

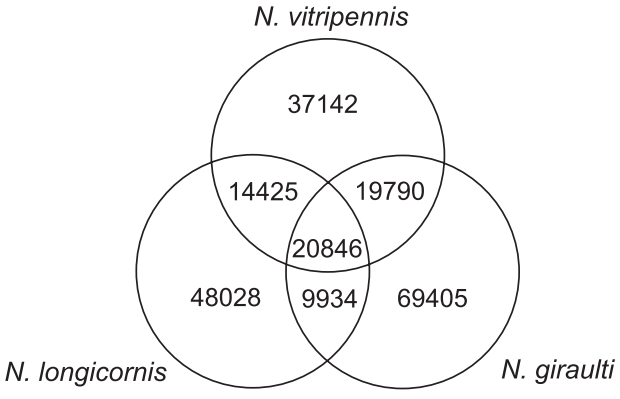

Figure 3. Venn diagram showing the overlap in microsatellites of three *Nasonia* species. The figures indicate the number of shared and unique microsatellites amongst species. The *Nasonia vitripennis* genome had a sixfold coverage, whereas the *Nasonia giraulti* and *Nasonia longicornis* genomes had a single-fold coverage.

The alternative, a higher mutation rate for *Nasonia*, seems to be less likely. In a recent study of mutation accumulation in offspring sex ratios in *N. vitripennis*, Pannebakker *et al.* (2008) found no obvious difference in mutation rate for this trait compared to similar life-history traits in other taxa. Whether DNA repair mechanisms actually show a reduced efficiency in Hymenoptera and other

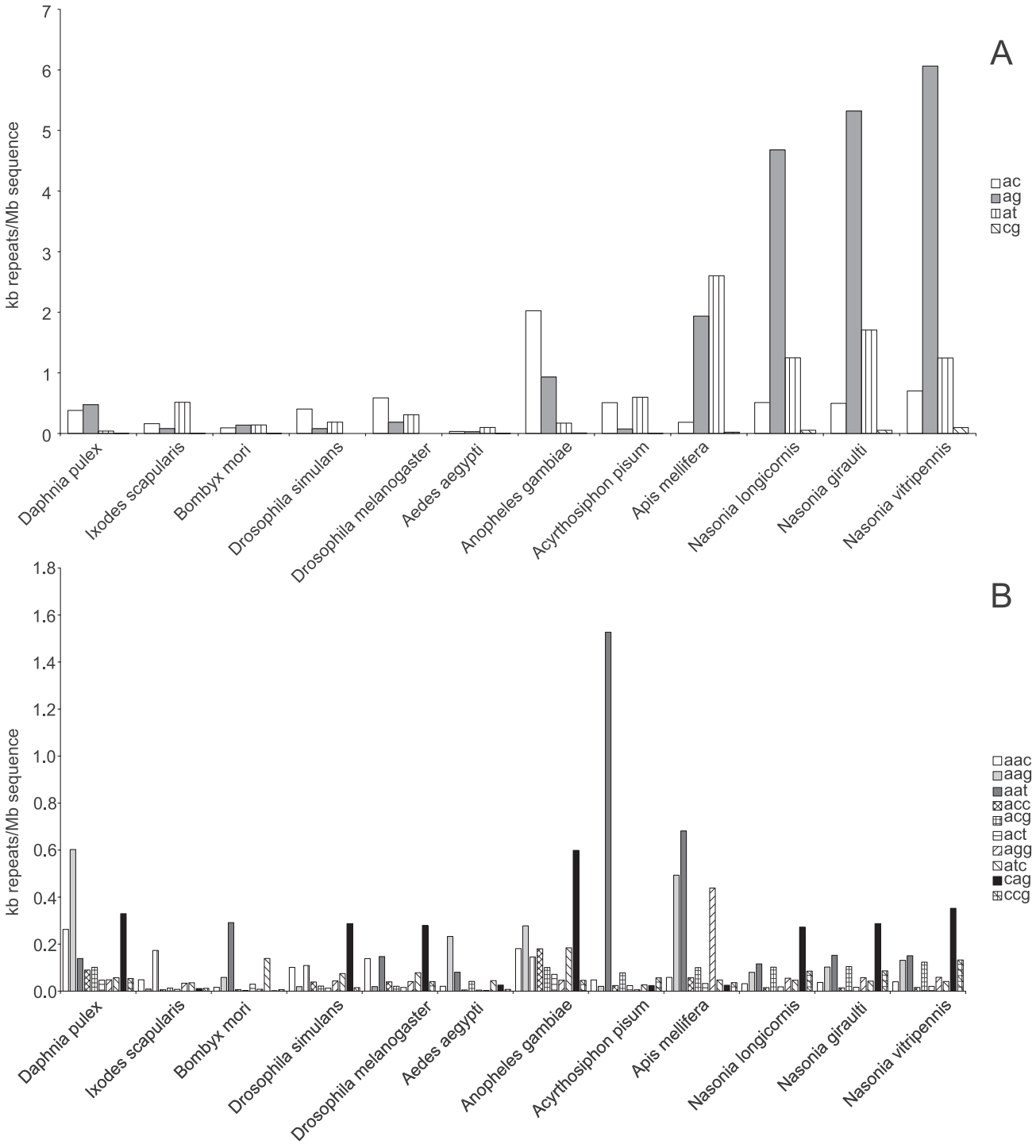

Figure 4. Motif type abundance for dinucleotide (A) and trinucleotide (B) repeats (in kb repeat sequence per Mb of analysed DNA) for the 12 studied arthropod genomes.

haplodiploids, thereby explaining the observed high abundance of repetitive DNA, remains a hypothesis to be tested.

The overall picture emerging from our data is one of heterogeneity in microsatellite abundance and composition across arthropod taxa. This pattern is corroborated by other studies into genomic microsatellite patterns in insects (Ross *et al.*, 2003; Archak *et al.*, 2007; Meglécz *et al.*, 2007) and on a wider range of eukaryotes (La Rota *et al.*, 2005; Sharma *et al.*, 2007) and prokaryotes

(Kassai-Jager *et al*., 2008). Even though the results of individual *in silico* studies can vary as a result of different thresholds for the inclusion of repeats, the observed pattern of heterogeneity is robust. The challenge for evolutionary geneticists is to explain this heterogeneity. The general consensus has been that factors such as differences in recombination may not explain differences in microsatellite distribution and diversity, with microsatellite loci playing a causal role in recombination hot spots rather than being a consequence of them (Ellegren, 2004; but see Bagshaw *et al*., 2008). The data presented here show heterogeneity in microsatellite density across the five chromosomes of *N. vitripennis*. In a recent study of recombination rates across the *N. vitripennis* genome, Niehuis *et al*., 2010 found a correlation between the presence of simple repeats (including monomers in that study) and the recombination rate, but again the direction of causation is far from certain. However, even though the observed patterns of microsatellite abundance and composition across taxa appear complex, the possibility of uncovering the biological basis of differences amongst species in microsatellite evolution may not be hopeless. For example, with more datasets becoming available, we may be able to test the extent to which species with different patterns or mechanisms of DNA mismatch repair vary in microsatellites. Moreover, a recent study has shown an association between body temperature and maximum microsatellite length in mammals (Amos & Clarke, 2008), suggesting that broad patterns might yet be awaiting discovery.

Experimental procedures

We obtained genome sequences for the three *Nasonia* species, *N. vitripennis*, *N. longicornis* and *N. giraulti* (Hymenoptera: Chalcidoidea: Pteromalidae), and for nine other arthropod species with published genome sequences: the honey bee, *A. mellifera* (Hymenoptera: Apoidea: Apidae), the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphidoidea: Aphididae), the yellow fever mosquito *Aedes aegypti* (Diptera: Culicoidea: Culicidae), the African malaria mosquito *Anopheles gambiae* (Diptera: Culicoidea: Culicidae), the fruit flies *Drosophila melanogaster* and *Drosophila simulans* (Diptera: Ephydroidea: Drosophilidae), the silkworm *Bombyx mori* (Lepidoptera: Bombycoidea: Bombycidae), the deer tick *Ixodes scapularis* (Acari: Ixodidae), and the water flea *Daphnia pulex* (Diplostraca: Daphniidae). Details of the assembly versions and sources are given in Supporting Information Table S1.

The genome sequences were scanned for the presence of microsatellites using MSATFINDER 2.09 (Thurston & Field, 2005) using the iterative search engine and allowing for interrupted microsatellites. In MSATFINDER, microsatellites are joined together in a single interrupted microsatellite when the distance between one microsatellite and the preceding one is equal to or less than the footprint of the current or preceding microsatellite and both share the same type of motif. We searched for microsatellites with motif length of two to six base pairs (bp) and minimum repeat numbers of eight, five, five, five and five (di-, tri-, tetra, penta- or hexanucleotides). For *N. vitripennis*, the microsatellites are available online through Genboree at Baylor College of Medicine (http://www.genboree.org/java-bin/login.jsp; database: Nasonia 1.0; track: seq: microsat).

The genome sequences used are all assembled into scaffolds or chromosomes, apart from the *N. longicornis* and *N. giraulti* sequences that are available only as single pass trace sequences. As this can result in a redundant detection of microsatellites, we clustered identical microsatellites of all three *Nasonia* species using MEGABLAST [parameters: filtering (-F): T; gap cost (-G): 1; gap extension (-E): -1; reward (-r): 1; wordsize (-W): 28; mismatch penalty (-q): -3; dropoff (-y): 10; final dropoff (-Z): 50; expect value (-e): $1e^{-5}$]. Clustered sequences were then assembled into consensus sequences using PHRAP (-forcelevel 10; Green, 1999).

Local microsatellite abundance was determined using the approach outlined by Niehuis *et al.* (submitted). Briefly, in a high-density *Nasonia* linkage map (1255 single nucleotide polymorphisms, sequence tagged sites (STS), or length polymorphic markers) spanning 446.9 cM, 264 marker clusters were defined by the absence of recombination between the markers (Niehuis *et al*., submitted). Average marker cluster size was 1.69 cM. For each marker cluster, microsatellite densities were calculated using the online version of MSATFINDER (Thurston & Field, 2005) with search parameters as indicated above.

Orthologous microsatellites within the *Nasonia* genus were identified using reciprocal BLASTN searches amongst the three species [parameters: filtering (-F): T; gap cost (-G): 2; gap extension (-E): 1; reward (-r): 1; wordsize (-W): 11; mismatch penalty (-q): -1; dropoff (-y): 100; final dropoff (-Z): 100; expect value (-e): $1e^{-5}$]. Redundant BLAST hits were filtered out using an in-house developed PERL script, and the intersects between the resulting datasets (*Nv*/*Ng*, *Nv*/*Nl* and *Nl*/*Ng*) were determined using the *merge* command in the R 2.8.0 software package (Ihaka & Gentleman, 1996; R Development Core Team, 2008).

Acknowledgements

We would like to thank Mark Blaxter and the members of the IEB Perl group for generous and fruitful discussion, the Nasonia Genome Sequencing Consortium, the Aphid Genome Sequencing Consortium, the Daphnia Genomics Consortium and the *Ixodes scapularis* Genome Project for making their data publicly available, and the Baylor College of Medicine-Human Genome Sequencing Center for providing the *Nasonia* and aphid sequence data, the US Department of Energy Joint Genome Institute for providing the *Daphnia* data, and the Broad Institute and the J. Craig Venter Institute (JCVI) for providing the *Ixodes* data. B. A. P. and D. M. S. were supported by the Natural Environment Research Council. B. A. P. acknowledges the Netherlands Organization for Scientific Research NWO (Veni grant 863.08.08) for funding. O. N. acknowledges the Alexander von Humboldt Foundation for a Feodor Lynen Research Fellowship for Postdoctoral Researchers.

Conflicts of interest

The authors have not declared any conflicts of interest.

References

Amos, W. and Clarke, A. (2008) Body temperature predicts maximum microsatellite length in mammals. *Biol Lett* **4**: 399–401.

Archak, S., Meduri, E., Kumar, P.S. and Nagaraju, J. (2007) InSatDb: a microsatellite database of fully sequenced insect genomes. *Nucleic Acids Res* **35**: D36–D39.

Ashley, C. and Warren, S. (1995) Trinucleotide repeat expansion and human disease. *Annu Rev Genet* **29**: 703–728.

Avise, J.C. (2004) *Molecular Markers, Natural Histology, and Evolution*, 2nd edn. Sinauer Associates, Sunderland, MA, USA.

Bagshaw, A., Pitt, J. and Gemmell, N. (2008) High frequency of microsatellites in *S. cerevisiae* meiotic recombination hotspots. *BMC Genomics* **9**: 49.

Beukeboom, L.W. and Desplan, C. (2003) *Nasonia*. *Curr Biol* **13**: R860.

Beukeboom, L.W., Niehuis, O., Pannebakker, B.A., Koevoets, T., Gibson, J.D., Shuker, D.M., van de Zande, L. and Gadau, J. (2010) A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of *Nasonia*. *Heredity*, doi:10.1038/hdy.2009.185.

Breeuwer, J.A.J. and Werren, J.H. (1995) Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution* **49**: 705–717.

Brent, A.E., Yucel, G., Small, S. and Desplan, C. (2007) Permissive and instructive anterior patterning rely on mRNA localization in the wasp embryo. *Science* **315**: 1841–1843.

Burton-Chellew, M.N., Koevoets, T., Grillenberger, B.K., Sykes, E.M., Underwood, S.L., Bijlsma, K. *et al.* (2008) Facultative sex ratio adjustment in natural populations of wasps: cues of local mate competition and the precision of adaptation. *Am Nat* **172**: 393–404.

Buschiazzo, E. and Gemmell, N. (2006) The rise, fall and renaissance of microsatellites in eukaryotic genomes. *Bioessays* **28**: 1040–1050.

Campbell, B.C., Steffen-Campbell, J.D. and Werren, J.H. (1993) Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Mol Biol* **2**: 225–237.

Cook, J.M. (1993) Sex determination in the Hymenoptera – a review of models and evidence. *Heredity* **71**: 421–435.

Crozier, R.H. (1977) Evolutionary genetics of hymenoptera. *Annu Rev Entomol* **22**: 263–288.

Crozier, R.H. (1985) Adaptive consequences of male-haploidy. In *Spider Mites. Their Biology, Natural Enemies and Control* (Helle, W. and Sabelis, M.W., eds), pp. 201–222. Elsevier, Amsterdam.

Ellegren, H. (2004) Microsatellites: simple sequences with complex evolution. *Nat Rev Genet* **5**: 435–445.

Ellison, C.K., Niehuis, O. and Gadau, J. (2008) Hybrid breakdown and mitochondrial dysfunction in hybrids of *Nasonia* parasitoid wasps. *J Evol Biol* **21**: 1844–1851.

Erickson, D.L., Fenster, C.B., Stenoien, H.K. and Price, D. (2004) Quantitative trait locus analyses and the study of evolutionary process. *Mol Ecol* **13**: 2505–2522.

Gadau, J., Werren, J.H. and Page, R.E. Jr (1999) Mapping of hybrid incompatibility loci in *Nasonia*. *Genetics* **153**: 1731–1741.

Gadau, J., Page, R.E. and Werren, J.H. (2002) The genetic basis of the interspecific differences in wing size in *Nasonia* (Hymenoptera; Pteromalidae): major quantitative trait loci and epistasis. *Genet* **161**: 673–684.

Gibson, J.D., Niehuis, O., Verrelli, B.C. and Gadau, J. (2010) Contrasting patterns of selective constraints in nuclear encoded genes of the oxidative phosphorylation pathway in holometabolous insects and their possible role in hybrid breakdown in *Nasonia*. *Heredity*, doi:10.1038/hdy.2009.172.

Goldstein, D.B. and Schlötterer, C. (1999) *Microsatellites: Evolution and Applications*, 1st edn. Oxford University Press, Oxford, UK.

Green, P. (1999) Phrap. http://phrap.org.

Gregory, T.R. (2006) Animal genome size database. http://www.genomesize.com.

Grillenberger, B.K., Koevoets, T., Burton-Chellew, M.N., Sykes, E.M., Shuker, D.M., De Zande, L. *et al.* (2008) Genetic structure of natural *Nasonia vitripennis* populations: validating assumptions of sex-ratio theory. *Mol Ecol* **17**: 2854–2864.

Henter, H.J. (2003) Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evology* **57**: 1793–1803.

Hirschhorn, J. and Daly, M. (2005) Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* **6**: 95–108.

Ihaka, R. and Gentleman, R. (1996) R: a language for data analysis and graphics. *J Comput Graph Stat* **5**: 299–314.

Kassai-Jager, E., Ortutay, C., Toth, G., Vellai, T. and Gaspari, Z. (2008) Distribution and evolution of short tandem repeats in closely related bacterial genomes. *Gene* **410**: 18–25.

Knott, S. (2005) Regression-based quantitative trait loci mapping: robust, efficient and effective. *Philos Trans R Soc Lond B Biol Sci* **360**: 1435–1442.

La Rota, M., Kantety, R.V., Yu, J.K. and Sorrells, M.E. (2005) Nonrandom distribution and frequencies of genomic and EST-derived microsatellite markers in rice, wheat, and barley. *BMC Genomics* **6**: 23.

Lynch, J.A., Brent, A.E., Leaf, D.S., Pultz, M.A. and Desplan, C. (2006) Localized maternal orthodenticle patterns anterior and posterior in the long germ wasp *Nasonia*. *Nature* **439**: 728–732.

Mackay, T.F.C. (2001) The genetic architecture of quantitative traits. *Annu Rev Genet* **35**: 303–339.

Meglécz, E., Anderson, S.J., Bourguet, D., Butcher, R., Caldas, A., Cassel-Lundhagen, A. *et al.* (2007) Microsatellite flanking region similarities among different loci within insect species. *Insect Mol Biol* **16**: 175–185.

Niehuis, O., Judson, A. and Gadau, J. (2008) Cytonuclear genic incompatibilities cause increase mortality in male F2 hybrids of *Nasonia girault*i and *Nasonia vitripennis*. *Genet* **178**: 413–426.

Niehuis, O., Gibson, J.D., Rosenberg, M., Pannebakker, B.A., Koevoets, T., Judson, A.K. *et al.* (2010). Recombination and its impact on the genome of the haplodiploid parasitoid wasp *Nasonia*. *PLOS One*, doi:10.1371/journal.pone.0008597.

Oda, S., Maehara, Y., Ikeda, Y., Oki, E., Egashira, A., Okamura, Y. *et al.* (2005) Two modes of microsatellite instability in human cancer: differential connection of defective DNA mismatch repair to dinucleotide repeat instability. *Nucleic Acids Res* **33**: 1628–1636.

Olesnicky, E.C. and Desplan, C. (2007) Distinct mechanisms for mRNA localization during embryonic axis specification in the wasp *Nasonia*. *Dev Biol* **306**: 134–142.

Pannebakker, B.A., Halligan, D.L., Reynolds, K.T., Ballantyne, G.A., Shuker, D.M., Barton, N.H. *et al.* (2008) Effects of spontaneous mutation accumulation on sex ratio traits in a parasitoid wasp. *Evology* **62**: 1921–1935.

Pearson, C.E., Edamura, K.N. and Cleary, J.D. (2005) Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet* **6**: 729–742.

Pietsch, C., Rutten, K. and Gadau, J. (2004) Eleven microsatellite markers in *Nasonia*, ASHMEAD 1904 (Hymenoptera; Pteromalidae). *Mol Ecol Notes* **4**: 43–45.

Pultz, M.A. and Leaf, D.S. (2003) The jewel wasp *Nasonia*: querying the genome with haplo-diploid genetics. *Genesis* **35**: 185–191.

Pultz, M.A., Westendorf, L., Gale, S.D., Hawkins, K., Lynch, J., Pitt, J.N. *et al.* (2005) A major role for zygotic hunchback in patterning the *Nasonia* embryo. *Development* **132**: 3705–3715.

R Development Core Team (2008) *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Ross, C., Dyer, K., Erez, T., Miller, S., Jaenike, J. and Markow, T. (2003) Rapid divergence of microsatellite abundance among species of *Drosophila*. *Mol Biol Evol* **20**: 1143–1157.

Saul, G.B. and Kayhart, M. (1956) Mutants and linkage in *Mormoniella*. *Genet* **41**: 930–937.

Schlötterer, C. (2004) The evolution of molecular markers – just a matter of fashion? *Nat Rev Genet* **5**: 63–69.

Selkoe, K. and Toonen, R. (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol Lett* **9**: 615–629.

Sharma, P.C., Grover, A. and Kahl, G. (2007) Mining microsatellites in eukaryotic genomes. *Trends Biotechnol* **25**: 490–498.

Strand, M., Prolla, T., Liskay, R. and Petes, T. (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**: 274–276.

Thurston, M. and Field, D. (2005) Msatfinder: detection and characterisation of microsatellites. Distributed by the authors at http://www.genomics.ceh.ac.uk/msatfinder/. CEH Oxford, Oxford.

Väli, U., Einarsson, A., Waits, L. and Ellegren, H. (2008) To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? *Mol Ecol* **17**: 3808–3817.

Velthuis, B.J., Yang, W.C., van Opijnen, T. and Werren, J.H. (2005) Genetics of female mate discrimination of heterospecific males in *Nasonia* (Hymenoptera, Pteromalidae). *Anim Behav* **69**: 1107–1120.

Werren, J.H. (1993) The evolution of inbreeding in haplodiploid organisms. In *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Thornhill, N.W., ed.), pp. 42–59. University of Chicago Press, Chigaco, IL.

Werren, J.H., Richards, S., Desjardins, C.A., Niehuis, O., Gadau, J., Colbourne, J.K., *et al.* (2010) Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science*, doi:10.1126/science.1178028.

Whiting, A.R. (1967) The biology of the parasitic wasp *Mormoniella vitripennis*. *Q Rev Biol* **42**: 333–406.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI 10.1111/j.1365-2583.2009.00915.x

Table S1. Genome sequences used for microsatellite analyses. (Adams *et al.*, 2000; Clark *et al.*, 2007; Holt *et al.*, 2002; Nene *et al.*, 2007; Weinstock *et al.*, 2006; Xia *et al.*, 2008).

Table S2. Local microsatellite abundance in regions of the *Nasonia vitripennis* genome.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

