

**Microsatellites – powerful tools for genome mapping and
genome evolution – a case study on the insect *Bombus
terrestris* and other social Hymenoptera.**

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Eckart Stolle

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Gutachter

1. Prof. Dr. Dr. Robin F. A. Moritz, Martin-Luther Universität Halle-Wittenberg
2. Prof. Dr. Olav Rüppell, University of North Carolina, Greensboro, NC, USA
3. Prof. Dr. Matthew Webster, Uppsala University, Sweden

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Vorsitzender der Promotionskommission: Prof. Dr. Robert J. Paxton, Martin-Luther Universität Halle-Wittenberg

Zusammenfassung

Die vorliegende Arbeit beschäftigt sich mit DNA Elementen, die häufig und in ihrer Länge variabel in den meisten Genomen vorkommen, den Mikrosatelliten. Im ersten Teil der Arbeit werden neue Mikrosatelliten Loci für die Erdhummel *Bombus terrestris* charakterisiert, um in dieser und anderen Hummelarten genutzt zu werden. Im zweiten Teil werden diese und weitere Mikrosatelliten benutzt, um das Genom der Erdhummel zu kartieren. Es konnte eine saturierte genetische Karte für die 16 Kopplungsgruppen (Chromosomen) erstellt werden, die als Basis für Genkartierung, Genom-Assemblierung und für den Vergleich von Rekombinationsraten dient. In einer weiteren Studie wurde die evolutionäre Dynamik von Mikrosatelliten in Dipteren und Hymenopteren untersucht und verglichen, wodurch eine schnellere Genomevolution in letzteren ersichtlich wurde. Im letzten Teil wird eine neue Methode vorgestellt, die auf kleinen Next-Generation-Sequencing Plattformen SNPs Genotypisierung erlaubt.

Abstract

The presented thesis has its focus on common and in their length length variable DNA elements which are present in most genomes: the microsatellites. In the first part of the thesis, novel microsatellite loci for the buff-tailed bumblebee *Bombus terrestris* are developed and characterized to be available for this and other bumblebee species. In the second part, these and further microsatellites are used to map the genome of the buff-tailed bumblebee. It was possible to saturated the genetic map and detect all 16 expected linkage groups (chromosomes), which can serve as a basis for gene mapping, genome assembly and the comparison of recombination rates. In the next study, the evolutionary dynamics of microsatellites was investigated in Diptera and Hymenoptera. This showed a faster rate of genome evolution in the latter. In the last part a novel method for genotyping by sequencing on small scale next generation sequencing platform is presented.

Publikationsliste Dissertation Eckart Stolle

- Stolle E, Moritz RFA (2013): **RESTseq – Efficient Benchtop Population Genomics with RESTriction fragment SEQuencing.**
[PLoS ONE](#) 8(5): e63960. doi:10.1371/journal.pone.0063960
- Stolle E, Kidner JH, Moritz, RFA (2013): **Patterns of Evolutionary Conservation of Microsatellites (SSRs) Suggest a Faster Rate of Genome Evolution in Hymenoptera Than in Diptera.**
[Genome Biology and Evolution](#) 5 (1): 151-162. doi: 10.1093/gbe/evs133
- Stolle E, Wilfert L, Schmid-Hempel R, Schmid-Hempel P, Kube M, Reinhardt R, Moritz RFA (2011): **A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae.**
[BMC Genomics](#) 12:48. doi: 10.1186/1471-2164-12-48
- Stolle E, Rohde M, Vautrin D, Solignac M, Schmid-Hempel P, Schmid-Hempel R, Moritz RFA (2009): **Novel microsatellite DNA loci for *Bombus terrestris* (Linnaeus, 1758).**
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Cumulative Dissertation

Eckart Stolle

Microsatellites – powerful tools for genome mapping and genome evolution – a case study on the insect *Bombus terrestris* and other social Hymenoptera.

General Introduction

Hymenoptera and Eusociality

Hymenoptera – “bees, wasps and ants” – are the third largest order of insects in terms of species numbers (Grimaldi & Engel 2005), but reach an even higher proportion in terms of biomass, mainly attributed to species which evolved an eusocial lifestyle. “Social insects account for just 2% of all insect species, but more than half the total insect biomass” (Wilson & Southwood 1990). Ants alone weigh about as much as all human beings combined (Hölldobler & Wilson 1990). Of all eusocially organized organisms in general, the insect order Hymenoptera comprises the vast majority of the species by far. Today bees, wasps and ants are genera with most competitive species animal that dominate many terrestrial ecosystems, especially in tropical regions (Grimaldi & Engel 2005). They are the main predators of invertebrates, the main pollinators of flowering plants and, because of their abundance, a major food source for entomophagous animals (Wilson 1971). Their enormous success is largely attributed to extreme adaptations in behavior and morphology. Their large societies are headed by a single or just a few individuals (queens) which monopolize reproduction over the other members of the society (workers). Workers are typically behaviorally and morphologically very different from the queen caste and contribute in a seemingly altruistic fashion to the overall colony fitness. Workers usually refrain from reproduction in response to behavioral and chemical signals of the queen(s) and are typically sterile. However, the reproductive division of labor is not entirely absolute, and workers can occasionally evade the control of the queen (Bourke 1988; Ross & Matthews 1991; Peeters 1991; Bloch & Hefetz 1999; Peeters & Liebig 2000; Lopez-Vaamonde *et al.* 2009; Seeley 2010; Huth-Schwarz *et al.* 2011).

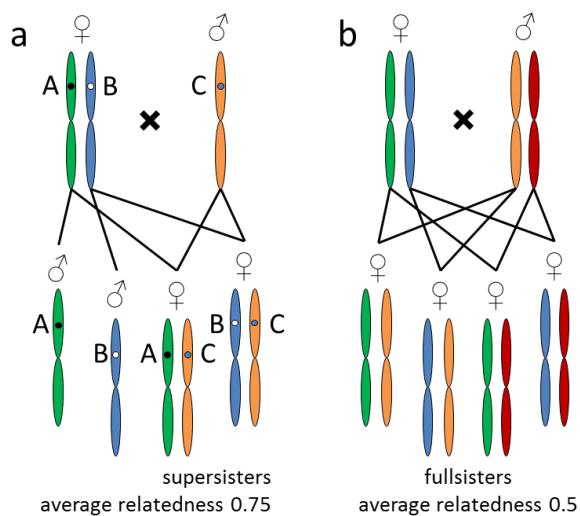
The regulation of these eusocial societies and the caste system is an extraordinary example of phenotypic plasticity. Worker and sexual offspring can have identical genetic information, yet they develop into individuals, which can be very different in behavior and morphology. Whereas primitively eusocial species usually show little caste differentiation, the highly advanced species like leaf cutter or army ants are most extreme with distinct sub-castes within the worker caste (Hölldobler & Wilson 1990; Hunt 2007; Jaffé *et al.* 2007; Seeley 2010).

The altruistic behavior of the worker caste and its regulation has gained considerable attention in scientific research. It is a puzzling issue as it seems to be in conflict with Darwin's theory of evolution by natural selection (Darwin 1859). However, no classical inclusive fitness theory plausibly explained how altruism can be an evolutionary stable strategy based on relatedness of the individuals in a colony (Hamilton 1964). Nevertheless, ever since this remained to be a controversially discussed subject (Queller *et al.* 1993; Queller & Strassmann 1998; Hunt & Amdam 2005; Hughes *et al.* 2008; Boomsma 2009; Nowak *et al.* 2010; Abbot *et al.* 2011; Strassmann *et al.* 2011; Boomsma *et al.* 2011; Ferriere & Michod 2011; Herre & Wcislo 2011; Gardner & Ross 2013). In spite of potential alternative explanations recent studies suggested that haplodiploidy and (ancestral) monogamy in Hymenoptera (Hughes *et al.*

2008; Gardner & Ross 2013) play a key role in the evolution and maintenance of sociality by enhancing relatedness among the offspring of an individual. This might have facilitated the repeated and independent evolution of sociality in several groups of Hymenoptera – wasps (Vespidae), ants (Formicidae) and several groups within the Apoidea (Crabronidae, Allodapidae, Halictidae, Apidae) (Hughes *et al.* 2008).

As mentioned above, Hymenoptera are haplodiploid insects in which fertilized eggs develop into diploid females and unfertilized haploid eggs develop into males (Figure 1a). In consequence, the female workers in a colony are super-sisters, unless the queen is multiply mated, as they possess the identical chromosomal set as the father. Thus, the relatedness among sibs is higher, namely 0.75 on the average, than it would be in diploid-diploid organisms offspring (Figure 1b).

Figure 1 crossing scheme for haplodiploid organisms with complementary sex determining locus (alleles A, B and C) **(a)** and diploid organisms **(b)**



Because only very few individuals reproduce eusocial Hymenoptera exhibit a low effective populations size (Zayed 2004; Bromham & Leys 2005; Jaffé *et al.* 2010). This results in increased genetic drift – a random loss of genetic diversity – playing a major role in the molecular evolution in these species. The reduced genetic variance should in turn reduce the effect of natural selection (Lynch 2007). One way to enhance the effective population size, genetic diversity, and thus also more efficient selection, is polygyny or polyandry, which is found in various ant and bee species (Hölldobler & Wilson 1990; Kraus *et al.* 2005; Hughes *et al.* 2008; Seeley 2010). Also an increase the recombination frequency can contribute to an enhanced genetic diversity (Charlesworth *et al.* 2009; Betancourt *et al.* 2009) which could be shown for various eusocial species, once the appropriate molecular tools had been developed (Beye *et al.* 2006; Wilfert *et al.* 2006; Sirviö *et al.* 2006, 2011; Weinstock *et al.* 2006).

A milestone was the comprehensive mapping and sequencing of the honeybee *Apis mellifera* genome in 2006 (Weinstock *et al.* 2006; Solignac *et al.* 2007). which triggered extensive research on genetic mechanisms underlying social behavior and its consequences in the rather new field of sociogenomics (Rueppell *et al.* 2006; Münch *et al.* 2008; Zayed & Whitfield 2008; Kucharski *et al.* 2008; Amdam *et al.* 2010; Graham *et al.* 2011; Flores & Amdam 2011; Havukainen *et al.* 2011; Page *et al.* 2012; Rangberg *et al.* 2012). Moreover, it was shown that this highly eusocial species exhibited an extremely high recombination rate, among the highest in the animal kingdom (Beye *et al.* 2006). Another particularity was a very high AT content of the genome and the low frequency of retro-transposable elements, only containing a small fraction of R2 long interspersed repeats (Weinstock *et al.* 2006). The substitution rates in orthologous protein coding genes suggested a slower genome evolution than in Diptera (Weinstock *et al.* 2006) which contradicted other findings (Bromham & Leys 2005). This is interesting since a high recombination rate and strong genetic drift should have a strong impact on genome evolution (Bromham & Leys 2005; Lynch 2007; Charlesworth *et al.* 2009; Charlesworth 2009).

The recent publication of draft whole genome sequences from several ant species (Bonasio *et al.* 2010; Suen *et al.* 2011; Smith *et al.* 2011a; b; Wurm *et al.* 2011; Nygaard *et al.* 2011; Gadau *et al.* 2012) and preliminary results from other bee species (Kim Worley *et al.*, unpublished, Baylor College of Medicine, pers. communication) so far indicated some commonalities among these species. Apart from high recombination rates – known for two ant and two bee species, they also exhibit high genomic AT contents. However, the majority of the sequenced Hymenoptera species belong to the ants, hence comparative results might be phylogenetically biased. Only one bee species was sequenced so far (*A. mellifera*) and although it is a valuable source for molecular data, it has certain limits. In many aspects, the honeybee is highly adapted and thus rather derived. Therefore any expansion of the molecular resources for additional bee species, especially those species which show a more primitive organization, would greatly facilitate our understanding of the mechanisms underlying genome evolution since it opens new possibilities for comparative sociogenomic analyses.

The bumblebee *Bombus terrestris*

Among the almost 250 species of bumblebees, the majority are primitively eusocial species (Williams 1998). Their usually annual colonies are independently founded by a single female. Compared to the advanced eusocial honeybee *Apis mellifera* or some stingless bee societies, bumblebee colonies are much smaller and differences between castes are much less prominent, often merely behavior based (Plowright & Lavery 1984; Goulson 2003).

Although bumblebees are globally distributed, the majority of species occurs in temperate or cold climates for which they seem to be well adapted. In many ecosystems they can be of great relevance for the reproductive success of flowering plants as they are efficient pollinators which are also active at low temperatures. Due to manmade changes in the landscape, many species are declining to the extent of becoming locally extinct (Darvill *et al.* 2006; Ellis *et al.* 2006; Goulson *et al.* 2008).

Two species, *Bombus terrestris* (Figure 2) and *B. impatiens* gained economic importance for their pollination service in crop production (Willmer *et al.* 1994; Morandin *et al.* 2001; Velthuis & van Doorn 2006). This and other aspects of their biology triggered a wide range of studies, especially on *B. terrestris* (Larrere & Couillaud 1993; Estoup *et al.* 1995a; Hoshiba *et al.* 1995; Goulson & Darvill 2004; Wilfert *et al.* 2007b; Petit *et al.* 2007; Kraus *et al.* 2009; Whitehorn *et al.* 2009; Schlüns *et al.* 2010). Their similar, but simpler organization and the relatively close phylogenetic position to the honeybees, makes the bumblebees an attractive emerging model system. This is reflected by initiated efforts to sequence the genomes of *B. terrestris* and *B. impatiens* (Kim Worley, Baylor College of Medicine & P Schmid-Hempel, ETH Zürich, pers. communication).

Figure 2 The bufftailed bumblebee *Bombus terrestris* (picture: M. Betley)



However, so far the availability of molecular resources for *Bombus terrestris* was limited. A number of molecular markers, RAPD, RFLP, AFLP and microsatellites (Estoup *et al.* 1993, 1995a; b, 1996; Gadau *et al.* 2001; Reber-Funk *et al.* 2006; Murray *et al.* 2008) had been available and were employed for

generating two coarse genetic maps (Gadau *et al.* 2001; Wilfert *et al.* 2006). Recently, an EST and a BAC library were published (Wilfert *et al.* 2009; Sadd *et al.* 2010).

The first genetic map for *B. terrestris* (Gadau *et al.* 2001) used RAPD markers to map the sex determining locus. The second map (Wilfert *et al.* 2006) used AFLP and a small number of SSR markers to map the genome in order to be able to allow subsequent QTL linkage studies. Both maps lacked completeness and compatibility to some extent. RAPD markers are in general notoriously difficult to reproduce and there is no sequence information associated with them. The second map did not cover the complete genome, indicated by 21 linkage groups for 18 chromosomes (Hoshihara *et al.* 1995). In addition the density of markers was low and the majority was AFLP markers, which, although having some sequence information from the primers, are less suitable for further analyses on a genomic level.

Thus it was highly desirable to generate a comprehensive genetic map for this emerging model species. This would provide a basis for more robust QTL linkage mapping with higher resolution, it would provide comprehensive insights into the genome organization and more interestingly, it would yield an estimate of the genome-wide recombination rate. Furthermore it will be valuable for supporting the ongoing genome sequence project. With a genetic map, generated with markers with sequence information, it is possible to place sequences, contigs or scaffolds on chromosomes and potentially orientate them. This allows a subsequent analysis of the relationship between genetic and physical distance on a chromosomal or local scale which might indicate recombination hot- or cold spots. It also allows for interspecies comparisons of structure and organization on a chromosomal or genomic level.

In this thesis, these issues were addressed by developing new microsatellite loci, presented by publication I, as molecular markers in a sufficiently high density to produce a map of the *B. terrestris* genome to unambiguously determine all chromosomal linkage groups, presented by publication II. In addition to supporting the assembly of the bumblebee genome, these markers also might prove useful for population genetic studies in *B. terrestris* or other bumblebee species. The aim is to create the first comprehensive genetic map for this species, to derive the recombination rate and to compare genome structures with other species. This map will be the basis for many further genetic studies including genome assembly, population genomics, quantitative linkage mapping and so forth.

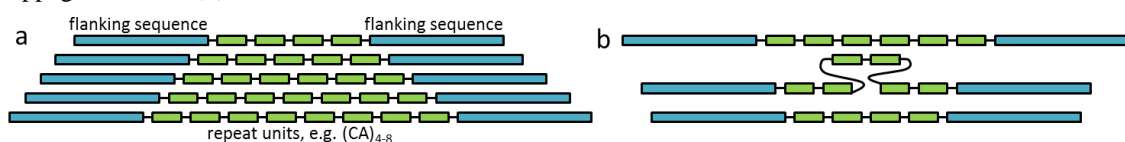
Mapping the bumblebee genome with microsatellite markers has the advantage of providing sequence information for each mapped marker. This way it is possible to find orthologous loci in other, related species' genomes such as the honeybee *Apis mellifera*, and investigate their positional relationships in order to shed light on their genome evolution. Orthologous loci further can be used to investigate evolutionary properties of microsatellites as such, since they provide information on characteristics, presence as such or absence.

Microsatellites and their evolutionary conservation

Microsatellites or simple sequence repeats (SSRs) have become state-of-the-art markers for a large number of studies in population genetics, QTL mapping, genome mapping, conservation genetics, mating biology and so forth (Estoup *et al.* 1995a; Solignac *et al.* 2004; Kraus *et al.* 2005; Lattorff *et al.* 2007; Wilfert *et al.* 2007a; Moritz *et al.* 2007; Jaffé *et al.* 2007, 2010).

Microsatellites are repetitive DNA sequences, small sequence motifs which are tandemly repeated. These motifs are usually 1 to 6 bp long and can be found in all eukaryotic organisms (Goldstein & Schlötterer 1999; Schlötterer 2000; Ellegren 2004). Their high within species variability and usefulness as a population genetic marker is owed to a high rate of slippage mutation which is frequently giving rise to multiple alleles of different length per locus (Figure 3a,b) (Leclercq *et al.* 2010).

Figure 3 alleles of a microsatellite with different numbers of repeat units (a), schematic of replication slippage mutation (b).



The widespread consensus is that these sequences are typically non-coding and selectively neutral. Their mutation rates are much higher than in other sequences, and thus it was expected, that these sequences have a high turnover during evolution. However, some recent studies could show that non-coding DNA sequences and also microsatellites can be conserved over evolutionary time scales (Keightley *et al.* 2005; Buschiazzo & Gemmell 2006, 2009, 2010; Sun *et al.* 2009). It is intriguing that such “neutral” sequences are retained in genomes for many million years of separate evolution. Although this was partially known for quite some time – indicated by successful cross-species usage of microsatellite loci (Vaiman *et al.* 1994; FitzSimmons *et al.* 1995; Primmer *et al.* 1996; Reber-Funk *et al.* 2006; Barbará *et al.* 2007) – only recently available genome scale data analyses comprehensively showed, that conservation of microsatellites can be substantial. But again, so far this has been shown only for a limited number of vertebrate species (Buschiazzo & Gemmell 2010).

For insects, no genome-wide studies on evolutionary conservation are available. This is partly attributed to the lack of genome sequences, which only became available very recently. Apart from the honeybee, the flour beetle and the silkworm, there were only some species of *Drosophila* and mosquitos which had been sequenced. But during the last few years, further draft genome sequences from *Drosophila* species and numerous Hymenoptera were published. The relatively closely related species among the Diptera and Hymenoptera, made it especially feasible for identifying and characterizing orthologous microsatellite loci. There was the expectation of rather short evolutionary lifespans of these ‘neutral’ markers. Surprisingly this had to be reconsidered after comparison of orthologous loci between *Bombus terrestris* and *Apis mellifera* (presented in publication II, as part of this thesis). Many microsatellite repeats were still present in both species, although they separated about 100 million years ago (Michener & Grimaldi 1988; Cameron & Mardulyn 2001; Grimaldi & Engel 2005; Danforth *et al.* 2006; Engel 2006; Hines 2008; Whitfield & Kjer 2008). Thus, a comprehensive analysis across many species will be most informative.

Microsatellites have high mutation rates (Schlötterer 2000; Leclercq *et al.* 2010) which gives rise to different alleles within short evolutionary times, hence their usefulness for discriminating individuals and populations. Although the dynamics of this variability is certainly interesting, it should reduce their value for studies over long evolutionary timescales (Buschiazzo & Gemmell 2009; Sun *et al.* 2009). However, the presence or absence of a microsatellite repeat as such, irrespective of its allelic state, could be used as a characteristic of genome evolution over longer evolutionary time scales.

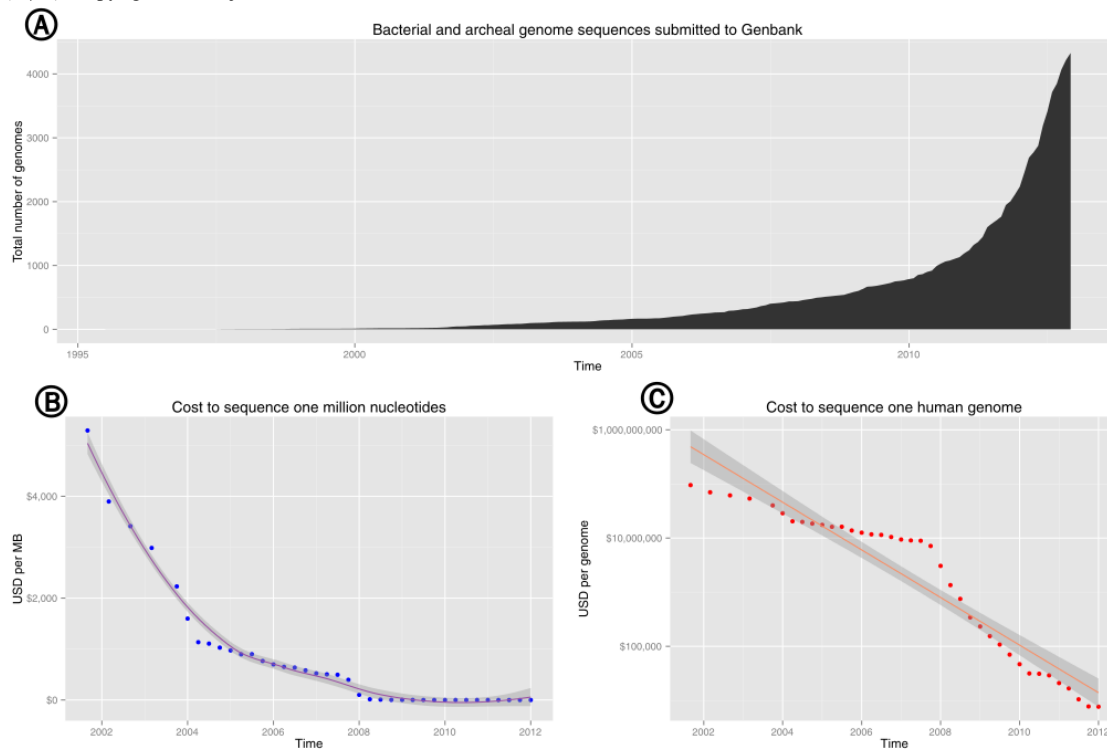
Whereas identifying microsatellites repeats is easy and fast (Benson 1999; Mayer *et al.* 2010; Mayer 2011), finding orthologous loci is more of a challenge. In a recent study on vertebrates, homologous regions between genomes were used to scan for repeats (Buschiazzo & Gemmell 2010). Such data are not available for insects, thus, a novel approach is necessary. A method independent from previous knowledge of homology would further reduce a potential bias in the analyses, since such conserved regions might be under certain selective constraints and hence lead to less reliable conclusions. Apart from helping to find and understand evolutionary constraints and patterns for microsatellite repeats, these orthologous loci could be exploited as a source for comparative genomics. First, rates of the loss of repeats, derived from the amount of conserved loci, could be used to infer and compare rates of genome evolution. Second, they could be used as a toolkit for studying genome-wide structural differences between species, synteny of genomic regions or be employed for genome sequence assembly improvements by comparing closely related species.

In the third publication of this thesis, these issues were addressed by developing a novel approach to identify and compare conserved microsatellites in several Diptera and Hymenoptera genomes. This was then used to compare rates of genome evolution and to find patterns of conservation for certain types of these repeats. Dated phylogenies were used for the species used in this study, to relate the conservation of microsatellites to divergence time. The latter aspect is particularly important as it is a potential source of erroneous results. Species might differ considerable in certain biological aspects, thus molecular clocks might not tick at uniform rates (cf. Bromham & Leys 2005), thus the number of evolutionary relevant events might differ per unit time. In consequence, additionally to the genomic information life history data were used to correct the datasets on rates of evolution, an asset which has not been explored in many such studies, although it might provide a more complete view on the subject.

Alternative molecular markers and new technologies

Microsatellites undoubtedly had a huge impact on molecular ecology and genetic studies on a population and genomic scale. However, new sequencing technologies have been developed and these next generation or high throughput systems reduced the expense of sequencing dramatically (Metzker 2010; Mardis 2011; Glenn 2011; Quail *et al.* 2012) (Figure 4b,c). Their further refinement and improved capacity and efficiency, the development of new bioinformatics algorithms for data analyses and exploding usage of next generation sequencing technologies (Figure 4a) revolutionized the field of molecular ecology, population genetics and genomics. Studies can now densely cover whole genomes with single nucleotide polymorphisms (SNPs) and/or process large numbers of samples. This opened many new avenues for more comprehensive and previously hardly possible studies. An example would be whole genome re-sequencing for population genetic analysis of whole genomes on a base-pair resolution to extract regions of balancing or purifying selection, selective sweeps, QTLs, whole genome associations, copy number variation, transposable element activity, recombination breakpoints or genome rearrangements.

Figure 4 Exponential increasing number of finished genome sequences (A) and the cost of sequencing (B,C) (copyright Estevezj, Wikimedia Commons)



However, if genomes are too large, whole genome sequences are not available or high resolution is combined with large numbers of desired markers or samples, it is still a challenge to conduct such projects both in terms of labor and a financial intensity. For many studies at the population genomic level it is sufficient have highly reduced information on the genome. Markers are needed that saturate the genome at sufficient density to reveal all coding regions in the genome. In order to lower the complexity of a genome – to get a reduced representation of a genome – and thus decreasing the amount of DNA to be sequenced, various methods to prepare the sequencing libraries have been developed (Van Orsouw *et al.* 2007; Tassell *et al.* 2008; Baird *et al.* 2008; Andolfatto *et al.* 2011; Elshire *et al.* 2011; Peterson *et al.* 2012; Wang *et al.* 2012). All of them are based on the use of restriction endonucleases and/or size fractioning. Although they were successfully used in numerous studies (Davey *et al.* 2011; Luca *et al.* 2011; Baxter *et al.* 2011; Ma *et al.* 2012; Wang *et al.* 2013), the sequencing effort remains still rather high, especially to cover certain shared regions in all samples. For many studies, especially in population genetics and molecular ecology, high numbers of markers are not essential, but rather require a large sample size. In addition, new benchtop-type next generation sequencing machines (e.g. Ion Torrent,

MiSeq) have limited capacity and thus are not well suited for methods based on large genome libraries (Loman *et al.* 2012). The small platform would profit if we could enrich the informative markers in the libraries to be screened.

To overcome these shortcomings, a new method of library preparation was developed as part of this thesis to meet requirements of research studies, as well as adapt to the capacities of benchtop sequencers (although this does not exclude large scale platforms). Using simple and well established techniques, now the complexity of genomic libraries can be reduced reliably. The method generates a reduced representation of the genome which generates sequences distributed over a genome without bias and in a reproducible fashion so that a large overlap between samples can be gained. It is very flexible and can easily be adapted to the requirements of the study, whether it is low numbers of sequences for simple population genetic analyses or genome scale studies. Although it has similarities with other techniques, the advantages in this new approach might make this a valuable method for future research in the whole field of population genetics and genomics.

Study questions (papers)

- I. Development and evaluation of new microsatellite loci as molecular markers in *Bombus terrestris* and other bumblebees.
- II. Generation of a genetic linkage map for the *B. terrestris* genome with a sufficient number of markers to saturate it, determine recombination rate, and compare the genome structures with the related species *Apis mellifera*, for which a genetic linkage map is available as well.
- III. Comparative study on the evolutionary conservation of microsatellites in insect genomes, identification possible patterns and comparison of rates of genome evolution in Hymenoptera and Diptera.
- IV. Development of a new method to adapt next generation sequencing, especially with benchtop machines, for genome and/or population scale identification and genotyping of SNPs as alternative molecular markers for population genetics on large sample numbers or genome scale mapping.

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I. Novel microsatellite DNA loci for *Bombus terrestris* (Linnaeus, 1758).

Eckart Stolle^{1*}, Mandy Rohde¹, Dominique Vautrin², Michel Solignac², Paul Schmid-Hempel³, Regula Schmid-Hempel³, Robin F. A. Moritz¹

¹ Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, D-06099 Halle (Saale), Germany.

² Laboratoire Evolution, Génomes et Spéciation, CNRS, 91198 Gif-sur-Yvette cedex, France.

³ ETH Zürich, Institute for Integrative Biology (IBZ), Experimental Ecology Group, ETH-Zentrum CHN, CH-8092 Zürich, Switzerland.

* Correspondence: Eckart Stolle, Fax: + +49-345-5527264; E-mail: eckart.stolle@zoologie.uni-halle.de

Abstract

We present details and characteristics of 123 novel polymorphic microsatellite DNA loci for *Bombus terrestris*. Thirty-four of these loci have been tested in nine other *Bombus* species and 25 of them showed polymorphisms in at least one species. These microsatellite DNA loci together with the already established 60 loci will be useful for characterizing wild and managed populations of *B. terrestris* and other *Bombus* species as well as for detailed genetic studies including mapping studies and genome annotations.

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II. A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae.

Eckart Stolle^{1*}, Lena Wilfert^{2,4}, Regula Schmid-Hempel², Paul Schmid-Hempel², Michael Kube³, Richard Reinhardt^{3,5}, Robin F. A. Moritz¹

¹ Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, D-06099 Halle (Saale), Germany.

² Institute of Integrative Biology (IBZ), ETH Zürich, Universitätsstrasse 16, CH-8092 Zürich, Switzerland.

³ Max Planck Institute for Molecular Genetics, Ihnestraße 63-73, D-14195 Berlin, Germany.

⁴ Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK.

⁵ Genome Centre Cologne at MPI for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany.

* Correspondence: eckart.stolle@zoologie.uni-halle.de

Abstract

Background: The bumblebee *Bombus terrestris* is an ecologically and economically important pollinator and has become an important biological model system. To study fundamental evolutionary questions at the genomic level, a high resolution genetic linkage map is an essential tool for analyses ranging from quantitative trait loci (QTL) mapping to genome assembly and comparative genomics. We here present a saturated linkage map and match it with the *Apis mellifera* genome using homologous markers. This genome-wide comparison allows insights into structural conservations and rearrangements and thus the evolution on a chromosomal level.

Results: The high density linkage map covers ~ 93% of the *B. terrestris* genome on 18 linkage groups (LGs) and has a length of 2'047 cM with an average marker distance of 4.02 cM. Based on a genome size of ~ 430 Mb, the recombination rate estimate is 4.76 cM/Mb. Sequence homologies of 242 homologous markers allowed to match 15 *B. terrestris* with *A. mellifera* LGs, five of them as composites. Comparing marker orders between both genomes we detect over 14% of the genome to be organized in synteny and 21% in rearranged blocks on the same homologous LG.

Conclusions: This study demonstrates that, despite the very high recombination rates of both *A. mellifera* and *B. terrestris* and a long divergence time of about 100 million years, the genomes' genetic architecture is highly conserved. This reflects a slow genome evolution in these bees. We show that data on genome organization and conserved molecular markers can be used as a powerful tool for comparative genomics and evolutionary studies, opening up new avenues of research in the Apidae.

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III. Patterns of evolutionary conservation of microsatellites (SSRs) suggest a faster rate of genome evolution in Hymenoptera than in Diptera.

Eckart Stolle^{1*}, Jonathan H. Kidner¹, and Robin F. A. Moritz^{1,2}

¹ Department of Zoology, Institute of Biology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

² Department of Zoology and Entomology, University of Pretoria, Pretoria 0002 South Africa

* Corresponding author: E-mail: eckart.stolle@zoologie.uni-halle.de.

Abstract

Microsatellites, or simple sequence repeats (SSRs), are common and widespread DNA elements in genomes of many organisms. However, their dynamics in genome evolution is unclear, whereby they are thought to evolve neutrally. More available genome sequences along with dated phylogenies allowed for studying the evolution of these repetitive DNA elements along evolutionary time scales. This could be used to compare rates of genome evolution. We show that SSRs in insects can be retained for several hundred million years. Different types of microsatellites seem to be retained longer than others. By comparing Dipteran with Hymenopteran species, we found very similar patterns of SSR loss during their evolution, but both taxa differ profoundly in the rate. Relative to divergence time, Diptera lost SSRs twice as fast as Hymenoptera. The loss of SSRs on the *Drosophila melanogaster* X-chromosome was higher than on the other chromosomes. However, accounting for generation time, the Diptera show an 8.5-fold slower rate of SSR loss than the Hymenoptera, which, in contrast to previous studies, suggests a faster genome evolution in the latter. This shows that generation time differences can have a profound effect. A faster genome evolution in these insects could be facilitated by several factors very different to Diptera, which is discussed in light of our results on the haplodiploid *D. melanogaster* X-chromosome. Furthermore, large numbers of SSRs can be found to be in synteny and thus could be exploited as a tool to investigate genome structure and evolution.

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IV. RESTseq – Efficient Benchtop Population Genomics with RESTriction fragment SEQuencing.

Eckart Stolle^{1*}, Robin F. A. Moritz^{1,3,4}

¹ Institute of Biology, Department of Zoology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

² Bio-Solutions GmbH, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

³ Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

⁴ Facultatea de Zootehnie și Biotehnologii, Universitatea de Științe Agricole și Medicină Veterinară, Cluj-Napoca, Romania

* corresponding author, E-Mail: eckart.stolle@zoologie.uni-halle.de

Abstract

We present RESTseq, an improved approach for a cost efficient, highly flexible and repeatable enrichment of DNA fragments from digested genomic DNA using Next Generation Sequencing platforms including small scale Personal Genome sequencers. Easy adjustments make it suitable for a wide range of studies requiring SNP detection or SNP genotyping from fine-scale linkage mapping to population genomics and population genetics also in non-model organisms. We demonstrate the validity of our approach by comparing two honeybee and several stingless bee samples.

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General Discussion

The bumblebee, *Bombus terrestris* became an emerging model system, and is the focus of many studies concerning most diverse biological aspects. The work presented in this thesis (Stolle *et al.* 2009, 2011), provided a significant contribution with respect to the genome of this species. The first saturated genetic linkage map for the genome of the buff-tailed bumblebee filled an important knowledge gap (Colgan *et al.* 2011; Schmieder *et al.* 2012; Kidner & Moritz 2013; Stolle *et al.* 2013; Leboeuf *et al.* 2013). *B. terrestris* is now the second bee species and one of the few Hymenopterans which have their genomes mapped (Sirviö *et al.* 2006, 2011b; Solignac *et al.* 2007; Werren *et al.* 2010). Apart from advances in coverage and density of the genetic map compared to previous, incomplete studies (Gadau *et al.* 2001; Wilfert *et al.* 2006), the usage of newly developed microsatellite marker provided the base tool kit for numerous further investigations. Not only can quantitative trait loci be mapped more precisely, the map and its markers proved most important to anchor and orientate sequences within the ongoing bumblebee genome project (Kim Worley, Baylor College of Medicine) also allowing for more robust estimates of the genomic recombination rate. Many of the novel markers have already been used in various subsequent studies of other species (An *et al.* 2011; Cook 2011; Huth-Schwarz *et al.* 2011a; b; Cerna & Straka 2012; Jha & Kremen 2013).

The sequence information from flanking regions of the microsatellites further allowed for other studies. Due to sequence similarities, orthologous loci were detected in the genome of the related honeybee and subsequently yielded the surprising result of a high degree of conservation of chromosomal structures (Stolle *et al.* 2011). While this is expected in closely related species, such a degree of conservation between the bumblebee and the honeybee was striking since both species separated about 100 million years ago. It was also striking that microsatellite repeats themselves were apparently conserved to some extent, given their neutral and highly mutative nature.

The comparative approach used in (Stolle *et al.* 2011) was adapted in (Stolle *et al.* 2013) for a comprehensive comparative study in several insect genomes, providing new insights about the evolutionary conservation of microsatellites. This showed for the first time that such putatively neutral sequences can be retained in invertebrate genomes for many million years. The latter fact made microsatellites a powerful tool for comparing rates of genome evolution in (mostly) social Hymenoptera and Diptera. An important aspect of the presented work and its findings was the consideration of life history characteristics of the analyzed species. Unlike in many other studies where rates of evolution are calculated based on universal molecular clocks, it was possible to show in the presented analysis, that biological traits can have a profound impact on the obtained results. After correcting for generation time, a characteristic for which data were available for all the species, the results of a much faster loss of microsatellite loci suggested a faster genome evolution in Hymenoptera. This result is likely to be more robust than previous estimates in which authors (including myself) did not include generation time and therefore came to opposite but probably less relevant conclusions (Weinstock *et al.* 2006; Stolle *et al.* 2011).

Social Hymenoptera are characterized by an extremely low effective populations size of an order of magnitude lower than in Diptera (*Drosophila*, *Aedes*, *Culex*, *Anopheles*) because only very few individuals reproduce. As a consequence it is expected that the resulting high genetic drift is causing an accelerated molecular evolution (Betancourt *et al.* 2002; Lynch 2007). This was actually proposed and shown in another study (Bromham & Leys 2005). However, microsatellites probably can be considered as largely neutrally evolving and thus have the advantage over coding sequences with selection constraints, to be less affected by genetic drift effects. Drift would act randomly on all alleles of a microsatellite, not biased towards specific allele lengths which should lead to the extinction of a microsatellite locus, thus drift could not explain the observed differences. The results from the study presented in publication III (Stolle *et al.* 2013) which built upon publications I and II (Stolle *et al.* 2009, 2011) provided the first empirical evidence based on putatively neutral markers to support a faster evolution in social species. The neutral markers are the main difference to the study of (Bromham & Leys 2005), in which other sequences were used and no correction for generation time was made. Despite similar conclusions, the results based on the microsatellites suggest that, although drift might influence molecular evolution of

coding regions, social Hymenoptera might have faster rates of genome evolution than other organisms due to other factors.

Some of the analyzed Hymenoptera evolved multiple mating probably also as a strategy to increase genetic variability at the colony and at the population level (Kraus *et al.* 2005; Jaffé *et al.* 2007; Rueppell *et al.* 2012). It is likely that this facilitates also higher efficacy of selection, which otherwise is expected to be low due to high genetic drift (Lynch 2007). However, this adaptation is only known for some social Hymenoptera species such as the honeybee (Kraus *et al.* 2005), others not (Schmid-Hempel & Schmid-Hempel 2000; Huth-Schwarz *et al.* 2011b). Since such a phenomenon would act on selection and drift as discussed above, it seems to be unlikely that it would have a large effect on the loss of neutral microsatellite markers.

Another factor which can influence different rates of genome evolution might be the recombination rate. High recombination rates have repeatedly been reported to be drivers for genome evolution (Hudson 1994; Lynch 2007; Mancera *et al.* 2008; Charlesworth *et al.* 2009; Betancourt *et al.* 2009; Kent *et al.* 2012). Empirical studies on genomic recombination frequencies are limited to a few species only thus far. However, a number of previous studies reported elevated rates for social insects compared to other non-social species (Beye *et al.* 2006; Sirviö *et al.* 2006, 2011a; b; Solignac *et al.* 2007; Wilfert *et al.* 2007; Meznar *et al.* 2010). As far as known, the honeybee exhibits the highest recombination rate in animal kingdom. With almost 16 cM/Mb it is an order of magnitude higher than in humans (0.6), nematodes (0.5) or fruit flies (3.8) (Lynch 2007; Wilfert *et al.* 2007). Other social species with such data available, two ants and a wasp, also have rates much higher than these non-socials. The results presented in this thesis (Stolle *et al.* 2011) further support the proposed relationship of sociality and elevated genomic recombination rates. For *Bombus terrestris*, if the genome size of 250 Mb from the final assembly (Kim Worley, Baylor College of Medicine) of the bumblebee genome is used, the estimate for the genomic recombination frequency is 8 cM/Mb.

It could thus be possible, that elevated recombination frequencies have evolved in social species to enhance selection efficacy in compensation for the small effective population sizes and high genetic drift (Hudson 1994; Lynch 2007; Mancera *et al.* 2008; Charlesworth *et al.* 2009; Betancourt *et al.* 2009; Kent *et al.* 2012). This is also supported by studies in *Drosophila* in which adaptations at the level of protein sequence (purifying selection or positive selection) is compromised in regions of low recombination (Presgraves 2005; Haddrill *et al.* 2007) such as the dot chromosome in *Drosophila* (Betancourt *et al.* 2009). Hence, although the explicit underlying mechanisms remain unclear, higher recombination rates provide an explanation for the difference between rates of genome evolution in Diptera and Hymenoptera as detected by neutral microsatellites.

However, despite such high recombination frequencies, the detected high level of structural conservation between genomes of the honeybee and bumblebee (Stolle *et al.* 2011) is still puzzling and unexpected. Elevated rates of recombination would be expected to elevate structural evolution. Why this was not observed in the bees is uncertain. It was reported for the genome of the honeybee that it contains an extremely low amount of transposable elements (Weinstock *et al.* 2006). In consequence, an impact of mobilized elements or inter-element recombination (due to high sequence similarity between copies of the same element) on chromosomal structure should be low and might offer an explanation for the structural conservation found (Lynch 2007; Novick *et al.* 2011).

The interplay of microsatellites with recombination rate, population structure and genome evolution, however, requires more detailed research in order to find correlative relationships and to unravel the highly complex molecular mechanisms underlying mutational and evolutionary processes of microsatellite repeats (Ellegren 2000). Yet a limited number of publications attempted to shed light on these interactions. For bacteria it was shown, that recombination lead to deletions in a tri-nucleotide microsatellite (Jakupciak & Wells 2000; Hashem *et al.* 2004). For birds it was found that mutational rates vary between sexes implying a potential influence from sexual selection, and that longer repeats are more prone to mutations (Anmarkrud *et al.* 2011). In humans, levels of slippage mutations leading to length polymorphism of microsatellite loci were shown to be not influenced by recombination rate (Payseur & Nachman 2000) but influenced by SNPs and InDels (Ellegren 2000; Brandström *et al.* 2008). In yeast, CA microsatellites were found to promote multiple cross-overs and to influence strand-exchange during recombination (Gendrel *et al.* 2000). In addition it was shown that there are differences between mitotic

and meiotic replication. The investigated microsatellites were less stable during meiosis. In conclusion, the knowledge about the molecular interplay of recombination and microsatellites is very incomplete. It appears to be important for future studies, to investigate and consider the influences of differences in mitotic and meiotic activities of germ-line cells and gametogenesis in males and females in multiple species on microsatellite and genome evolution. Such differences might have significant, thus far overlooked effects. This was already outlined by detected differences regarding male/female germ-line cell dynamics and their relation to the *Drosophila* X-chromosome evolution (Bauer & Aquadro 1997; Bachtrog 2008). And given the very long lifespans of the reproductive caste (queens) in social insects (cf. Page & Peng 2001; Lopez-Vaamonde *et al.* 2009; Parker 2010), the germ-cell line dynamics should receive more research attention.

Irrespective of the mechanisms behind different rates of genome evolution, the results presented in the publications of this thesis suggest a faster genomic evolution rate in social Hymenoptera than non-social species. It is proposed that recombination rate might play an important role. However, further studies are required and are under way in order to draw more comprehensive conclusions. Comparative analyses such as presented in this thesis, could greatly benefit from similar investigations in other organisms, social and non-social.

The technical advances in the recent few years open new opportunities for studying recombination and genome evolution in many more species. Although microsatellites are very robust and powerful molecular markers, especially for genetic linkage mapping as they exhibit high allelic variance, although they proved to be a very valuable tool in the presented studies (Stolle *et al.* 2009, 2011, 2013), they have limitations. Their development and utilization still requires much work and financial effort. With new methodological approaches such as presented in the last paper of this thesis (Stolle & Moritz), it is more feasible to use single nucleotide polymorphisms (SNPs) as alternative markers. By sequencing and irrespective of available sequence data, it is possible to generate and genotype SNPs in larger quantities at lower costs. This will greatly facilitate the analyses of many more species regarding recombination and rates of evolution und much shorter time.

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- Stolle E, Moritz RFA RESTseq – Efficient Benchtop Population Genomics with RESTriction fragment SEQuencing. *PlosOne*.

- Stolle E, Rohde M, Vautrin D, Solignac M, Schmid-Hempel P *et al.* (2009) Novel microsatellite DNA loci for *Bombus terrestris* (Linnaeus, 1758). *Molecular Ecology Resources*, **9**, 1345–1352.
- Stolle E, Wilfert L, Schmid-Hempel R, Schmid-Hempel P, Kube M *et al.* (2011) A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae. *BMC genomics*, **12**, 48.
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Appendix

Declaration on author contributions

Curriculum Vitae

Publication List

Eidesstattliche Erklärung

Declaration on author contributions

- **I.** Stolle E, Rohde M, Vautrin D, Solignac M, Schmid-Hempel P, Schmid-Hempel R, Moritz RFA (2009): Novel microsatellite DNA loci for *Bombus terrestris* (Linnaeus, 1758).

Molecular Ecology Resources 9(5): 1345-1352. DOI: 10.1111/j.1755-0998.2009.02610.x

I developed the majority of the novel microsatellites, the library production, the cloning, the sequencing, sequencing analyses and primer design and genotyping of individuals. About 10% of the final novel loci were provided by Mandy Rohde who performed her labwork in the labs of M. Solignac with assistance of D. Vautrin and in the lab of P. Schmid-Hempel in collaboration with R. Schmid-Hempel. M. Rohde also provided the Poland population sample. I did all the genotyping of the two populations and the cross-species testing as well as population genetics analysis and drafting the manuscript under the supervision of R. F. A. Moritz.

- **II.** Stolle E, Wilfert L, Schmid-Hempel R, Schmid-Hempel P, Kube M, Reinhardt R, Moritz RFA (2011): A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae.

BMC Genomics 12:48. DOI: 10.1186/1471-2164-12-48

I did the organization and labwork for this project and developed the additional markers, primer design, primer testing and genotyping for this work. I performed the complete analyses and drafted the paper under supervision of R. F. A. Moritz. L. Wilfert from P. Schmid-Hempels lab provided the mapping population and allowed with support by R. Schmid-Hempel a research visit of myself to extract the DNA. M. Kube and R. Reinhardt provided sequences from a previously generated BAC library, which was used to detect and develop further microsatellite loci.

- **III.** Stolle E, Kidner JH, Moritz, RFA (2013): Patterns of Evolutionary Conservation of Microsatellites (SSRs) Suggest a Faster Rate of Genome Evolution in Hymenoptera Than in Diptera.

Genome Biology and Evolution 5 (1): 151-162. DOI: 10.1093/gbe/evs133

I developed the initial concept, performed the data analyses and drafted the manuscript under supervision of R. F. A. Moritz. J. Kidner provided custom-made perl scripts for the analyses and supported the statistical analysis and contributed writing the manuscript.

- **IV.** Stolle E, Moritz RFA (in press): RESTseq – Efficient Benchtop Population Genomics with RESTriction fragment SEQuencing.

PlosOne.

I developed the initial concept and initial protocol, did the labwork and performed the analyses under supervision of R. F. A. Moritz.

Signatures,
30.04.2013

Halle,

E. Stolle

R. F. A. Moritz

J. H. Kidner

Curriculum Vitae

Personal Information

Name: Eckart Stolle

Birth: 16 January 1982, Sangerhausen, GDR

Nationality: German

Education

- Present-2007: Ph.D student at the Martin-Luther-University, Halle-Wittenberg, Germany. Dissertation Thesis concerned with "Microsatellites – powerful tools for genome mapping and genome evolution – a case study on the insect *Bombus terrestris* and other social Hymenoptera", supervised by Prof. Dr. Robin F. A. Moritz.
- 2007-2001: Diploma in Biology at the Martin-Luther-University, Halle-Wittenberg: "Molecular analysis of the candidate gene regulating parthenogenesis in honeybee workers", supervised by Prof. Dr. Robin F. A. Moritz.
- 2001-2000: civilian service (Zivildienst) "Naturschutzstation Südharz" (Außenstelle der Oberen Naturschutzbehörde Halle/S.) in Wippra (Harz)
- 2000: A-Level (Abitur) (Goethegymnasium Kelbra)

Courses, Internships, Research Visits

- 2012 Halle, Germany, Symposium on Advances and Challenges of RNA-Seq Analysis.
- 2010 (March): ETH Zürich, Switzerland (Experimental Ecology group of Paul Schmid-Hempel)
- 2010 Halle, Germany, Course "How to write DFG (German Science Foundation) research proposals" by SFI (collegiate initiative for promotion of nature sciences).
- 2010 Halle, Germany, Course "LaTeX" by SFI (collegiate initiative for promotion of nature sciences)
- 2009 (July): ETH Zürich, Switzerland (Experimental Ecology group of Paul Schmid-Hempel)
- 2009 (May): research visit in the lab of Yves Le Conte, INRA, Avignon (France) (funded by Prevention of honeybee Colony Losses (COLOSS))
- 2008 (July-Aug.): 11. Internationale Expedition „Kyrgyzstan 2008" (funded by the DaaD)
- 2008 (July): RethinkPCR – Scientific Conferences, Europe (Berlin, Germany)
- 2008 (May): ETH Zürich, Switzerland (Experimental Ecology group of Paul Schmid-Hempel)
- 2008 (Jan.- March): research visit at the University of Pretoria (South Africa) - Department Zoology & Entomology.
- 2006 (Jan.-July): research visit at the University of Pretoria (South Africa) - Department Zoology & Entomology (funded by the DaaD).
- 2005 Practical course on the expression of *csd* in *Apis mellifera* at the MLU, Institute for Zoology, lab of PD Dr. M. Beye (advisor Dr. M. Hasselmann)
- 2004 Practical course on the ant-repellent substance of the subsocial thrips *Suacerothrips linguis* at the MLU, Institute for Zoology, developmental biology and Thysanoptera research lab of Prof. Dr. G. Moritz (advisor Dr. G. Tschuch)
- 2003, Ilfeld, Germany, Practical course for Topographical Survey at Vermessungs-Service VSN Ilfeld GmbH
- 1999 IPK Gatersleben, Germany, Cytogenetic practical course at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, Cytogenetics lab of Prof. Dr. I. Schubert
- 1998 Practical course on plant secondary metabolites at The Federal Institute for Plant Breeding Research on crops, Institute for quality analytics, Quedlinburg, Germany

Work Experience

- Present-2011 FITBEE – national network project on honeybee health. Development of a quick detection assay for honeybee pathogens, especially viruses. BioSolutions GmbH (Halle), Project Supervision: Robin FA Moritz (MLU). Funded by BMfEL
- 2010-2007 DFG-funded project (“Mapping of the Bumblebee genome”), MLU, Institute for Zoology, molecular ecology lab, advisor Robin FA Moritz. Funded by DFG
- 2007-2005 Environmental Consultant (EVSA, Germany), FFH-species survey Saxony-Anhalt, Germany
- 2005 Student Research Assistant. Maintenance of *Drosophila melanogaster* mutant lines. MLU, Institute for Genetics, *Drosophila* developmental genetics and epigenetics lab of Prof. Dr. G. Reuter
- 2004 Student Research Assistant. Maintenance of locust populations (*Schistocerca gregaria*, *Locusta migratoria*) MLU, Institute for Zoology, animal physiology lab of Prof. Dr. H.-J. Ferenz
- 2003 Research Assistant. Sorting and Identification of entomological samples and development of a database for records of hoverflies (Diptera: Syrphidae). Environmental Research Centre (UFZ), Leipzig, Germany, Dr. F. Dziöck.
- 2002 Assignment: Database on hoverfly records in Saxony-Anhalt (Diptera: Syrphidae) Environmental Research Centre (UFZ), Leipzig, Germany, Dr. F. Dziöck
- 2002 Environmental Consultant (RANA, Halle) for two nature reserves (Diptera: Syrphidae and Hymenoptera)

Oral Presentations

- 2013 Queen Mary University London, lab of Yannick Wurm, invited seminar talk
- 2012 EurBee5 – European Conference of Apidology, Halle, Germany.
- 2012 Jahrestagung der Arbeitsgemeinschaft der Bieneninstitute Bonn, Germany.
- 2011 University Muenster, Institute for evolution and biodiversity, Prof. Erich Bornberg-Bauer: Evolutionary Bioinformatics, invited seminar talk
- 2011 International Social Insect Genomics Research Conference, BGI, Shenzhen, China
- 2011 Central European Meeting of the International Union for the Study of Social Insects – IUSI, Papenburg, Germany
- 2010 ETH Zürich, Switzerland, Experimental Ecology group of Prof. Paul Schmid-Hempel, invited seminar talk
- 2009 Jahrestagung der Arbeitsgemeinschaft der Bieneninstitute, Schwerin, Germany
- 2008 Herbsttagung im Biosphärenreservat Karstlandschaft Südharz, Rossla, Germany
- 2007 Jahrestagung der Arbeitsgemeinschaft der Bieneninstitute Veitshöchheim, Germany

Poster Presentations

- 2013 Tagung der Arbeitsgemeinschaft der Institute für Bienenforschung e.V. Würzburg, Germany
- 2012 Hym. Tagung, Stuttgart, Germany
- 2012 EurBee5 – European Conference of Apidology, Halle, Germany
- 2011 Central European Meeting of the International Union for the Study of Social Insects – IUSI, Papenburg, Germany.
- 2010 PhD Meeting of Evolutionary Biology of the DZG, Freiburg i.B., Germany
- 2010 Congress of the International Union for the Study of Social Insects IUSI, Copenhagen, Denmark
- 2009 Annual Meeting of Deutsche Zoologische Gesellschaft - DZG (Regensburg, Germany)
- 2009 New Molecular Tools Workshop, Bern, Switzerland

Miscellaneous

- Referee for *Heredity*, *Journal of Eukaryotic Microbiology*, *Insect Molecular Biology*.
- Part of the organization committee for EurBee5 – 5th European Conference of Apidology, 3-7th Sept 2012, Halle, Germany
- member of ESEB (European Society for Evolutionary Biology), IUSSI (International Union for the study of Social Insects, Central European Section), EVSA (Entomologen-Vereinigung Sachsen-Anhalt)
- editor of AMPULEX

Teaching and Mentoring

- 2013-2007 (co) supervision of students
Supervised >15 students during undergraduate and advanced practical courses, for research group practicals and research for two Bachelor thesis.
- present-2007 supervision and organization of student and public excursions
- present-2001 identification service as expert on certain groups of Hymenoptera.

Publication List

Peer reviewed

- Stolle E, Moritz RFA (2013): RESTseq – Efficient Benchtop Population Genomics with RESTriCTION fragment SEQUencing. **PLoS ONE** 8(5): e63960. DOI: 10.1371/journal.pone.0063960
- Stolle E, Kidner JH, Moritz, RFA (2013): Patterns of Evolutionary Conservation of Microsatellites (SSRs) Suggest a Faster Rate of Genome Evolution in Hymenoptera Than in Diptera. **Genome Biology and Evolution** 5 (1): 151-162. DOI: 10.1093/gbe/evs133
- Jarosch A, Stolle E, Crewe RM, Moritz RFA (2011): Alternative splicing of a single transcription factor drives selfish reproductive behavior in honeybee workers (*Apis mellifera*). **Proceedings of the National Academy of Sciences of the United States of America** 108 (37): 15282-15287. DOI: 10.1073/pnas.1109343108
- Stolle E, Wilfert L, Schmid-Hempel R, Schid-Hempel P, Kube M, Reinhardt R, Moritz RFA (2011): A second generation genetic map of the bumblebee *Bombus terrestris* (Linnaeus, 1758) reveals slow genome and chromosome evolution in the Apidae. **BMC Genomics** 12:48. DOI: 10.1186/1471-2164-12-48
- Jaffé R, Dietemann V, Allsopp MH, Costa C, Crewe RM, Dall’Olio R, de la Rúa P, El-Niweiri MAA, Fries I, Kezic N, Meusel MS, Paxton RJ, Shaibi T, Stolle E, Moritz RFA (2010): Estimating the Density of Honeybee Colonies across Their Natural Range to Fill the Gap in Pollinator Decline Censuses. **Conservation Biology** 24 (2): 583-593. DOI: 10.1111/j.1523-1739.2009.01331.x
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- Hoffmann MH, Bremer M, Schneider K, Burger F, Stolle E, Moritz G (2003): Flower visitors in a natural population of *Arabidopsis thaliana*. **Plant Biology** 5 (5): 491-494. DOI: 10.1055/s-2003-44784.

non-peer reviewed

- Gerth M, Franke F, Stolle E, Bleidorn C (2010): Ein neuer Nachweis der Goldwespe *Chrysis leachii* Shuckard, 1837 (Hymenoptera, Chrysididae) in Thüringen mit Anmerkungen zu potentiellen Wirten. **AMPULEX** 2: 61-64.
- Stolle E (2010): *Ectemnius fossorius* (Linnaeus, 1758) neu für Mecklenburg-Vorpommern (Hymenoptera, Crabronidae). **AMPULEX** 2: 75-76
- Peer Schnitter, Burkhard Lehmann, Gunthard Dornbusch, Katrin Hartenauer, Mathias Hohmann, Frank Meyer, Volker Neumann, Udo Richter, Wieland Röhricht, Peter Sacher, Karla Schneider, Andreas Schöne, Dietmar Spitzenberg, Eckart Stolle, Thoralf Sy, Michael Wallaschek & Werner Witsack (2007): Tierarten und Großpilze der Lebensraumtypen des Anhangs I der FFH-Richtlinie. **Naturschutz im Land Sachsen-Anhalt** 44 (2): 32-37.
- Stolle, E., Burger, F., Drewes, B. (2004): Rote Liste der Grabwespen (Hymenoptera: „Sphecidae“) des Landes Sachsen-Anhalt. – Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt 39 (2004): 369-375.
- Stolle, E., Burger, F. (2004): Rote Liste der Wegwespen, Spinnennameisen, Keulen-, Dolch- und Rollwespen (Hymenoptera: Pompilidae, Mutillidae, Sapygidae, Scoliidae,

Tiphiidae) des Landes Sachsen-Anhalt. – Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt 39 (2004): 375-381.

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- Stolle, E., Wolf, H. (2004): Fünf interessante Wegwespen-Arten Nordthüringens. - Aratora 14: 173-179. Artern.
- P. Schnitter, D. Frank, W. Bäse, R. Bartels, B. Billetoft, F. Burger, M. Dorn, G. Dornbusch, F. Gohr, C. Grosser, W. Gruschwitz, J. Haferkorn, D. Hanelt, M. Hohmann, U. G. Jäger, H. Jage, M. Jährling, M. Jentzsch, B. Kammerad, T. Karisch, W. Kleinsteuber, G. Körnig, C. Komposch, M. Lübke-Al Hussein, W. Malchau, F. Meyer, J. Müller, V. Neumann, B. Ohlendorf, J. Peterson, W. Röhrich, H. Ruhnke, P. Sacher, P. Schmidt, K. Schneider, P. Scholz, P. Scholze, P. Schütze, C. Schönborn, D. Spitzenberg, A. Stark, R. Steglich, E. Stolle, L. Tappenbeck, L. Täuscher, M. Trost, M. Wallaschek, W. Wendt, B. Winter-Huneck, W. Witsack, O. Wüstemann (2002): Auswahl der Arten der Lebensraumtypen nach Anhang I der FFH-Richtlinie. – In: Landesamt für Umweltschutz Sachsen-Anhalt (Hrsg.) (2002): Die Lebensraumtypen nach Anhang I der Fauna-Flora-Habitatrichtlinie im Land Sachsen-Anhalt. - Naturschutz im Land Sachsen-Anhalt 39, Sonderheft, 368 S.
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Eidesstattliche Erklärung

Halle, den 30.04.2013

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder bei der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg, noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich an Eides statt, dass ich dies Arbeit selbstständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Eckart Stolle