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Original article

Microsatellites for the inference of population structures in the Red Mason bee *Osmia rufa* (Hymenoptera, Megachilidae)[1]

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Abstract – Microsatellite loci were isolated from the solitary Red Mason bee (*Osmia rufa*) by an enrichment protocol for partial genomic libraries. Six polymorphic microsatellite loci were used for a first population structure survey including 9 continental European and one island population. Observed levels of genetic variability and heterozygosity proved to be moderate. There was no significant differentiation among continental *O. rufa* populations. Only the island population from Cyprus was clearly separated. A correlation between geographical and genetic distance indicates gene flow among continental bees. The relatively high homogeneity in *O. rufa* is probably caused by low effective population size rather than an imprint of past population events. All microsatellites amplified in three further *Osmia* species.

microsatellites / genetic diversity / population structure / *Osmia rufa*

1. INTRODUCTION

Solitary wild bees play an important role as pollinators of entomogamous plants in terrestrial ecosystems. They established a rich network of niche structures with diverse degrees of trophic specialisation reaching from oligolectic to polylectic species. However, number and diversity of solitary bees are dwindling in cultivated landscapes and they have become subject to increasing conservation efforts. On the other hand, there is a growing interest to establish wild bees as pollinators for crops (e.g. Parker et al., 1987; Richards, 1993; Seidelmann, 1993; Bosch and Kemp, 2002). As a consequence, domestic or semi-domestic populations with close genetic relatedness were transported over large distances and there is concern that autochthonous populations may become over flown with foreign geno- and ecotypes. Both the decline of the wild bee fauna as well as their swamping with semi-domesticated populations may cause a deterioration of the wild gene pool and hence a reduction of genetic fitness (inbreeding depression). It has been suggested that diminished genetic diversity and/or the disruption of adapted gene interactions may significantly impede the response to environmental changes (Frankel and Soulé, 1981; O'Brien et al., 1985; Packer and Owen, 2001) and therefore enhance the risk of extinction.

To develop conservation and management strategies for solitary wild bees, information on their genetic variation and population structure are essentially required. However, data on the genetic diversity of solitary bees are lacking both on a regional as well as an European scale.

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In general, there is profound evidence that Hymenopteran species exhibit relatively low levels of genetic polymorphisms (Blanchetot and Packer, 1992; Estoup et al., 1996; Roubik et al., 1996; McCorquodale and Owen, 1997), a phenomenon which is at least partially attributed to reduced effective population sizes caused by the haplo-diploid sex-determining mechanism. During the last decade, the use of variable microsatellites (e.g. Estoup et al., 1993, 1996; Strassmann et al., 1997) significantly increased the knowledge about population structure and mating strategies in Hymenopterans (e.g. Estoup et al., 1995; Paxton et al., 1996; Beye et al., 1998; Chapuisat, 1998; Beveridge and Simmons, 2004). However, genetic data concerning solitary bees are still extremely underrepresented although the vast majority of bees are not social.

In the following study we were aiming to isolate polymorphic microsatellite markers in the Red Mason bee (*Osmia rufa cornigera* (Rossi), syn: *O. bicornis*) to investigate the diversity and population structure of a representative solitary species. The ecology of this Megachilid has been well studied so far (Raw, 1972; Tasei, 1973; Maddocks and Paulus, 1987; Seidelmann, 1995) and the species is a prospective candidate for a managed pollinator in agriculture (Gladis, 1989; Roth, 1990; Wojtowski et al., 1995). The univoltine Red Mason bee is found throughout central Europe and large parts of southern and eastern Europe. The flight period lasts from mid April until mid June. Males emerge one to two weeks before females (protandry). *O. rufa* is polylectic and accepts a wide range of plants as food sources. Females of the Red Mason bee use a broad range of preexisting tube-like cavities to construct nests (typically beetle holes, empty hollow stalks). Such nest sites are usually scattered over the entire habitat and form only temporarily small agglomerations. *O. rufa* is philopatric and females prefer to breed in their natal nests or vacant holes nearby. Moreover, changing food resources and parasites, which regularly drive local nest agglomerations to extinction (Seidelmann 1995, 1999a), are responsible for large population fluctuations in a small regional scale (see e.g. Tepedino and Stanton, 1980; Tepedino and Parker, 1983, 1984; Parker, 1985 for other *Osmia* species). The sex ratio is regularly 1.5–3 fold male biased (reviewed by Seidelmann, 1995). Females mate only once (monandry) immediately after emergence. Male mating success is randomly distributed whereby only few males gain more than one copulation (max. 3–4). The majority of males fail to achieve any mating success (Seidelmann, 1999b).

2. MATERIALS AND METHODS

2.1. Isolation of microsatellites

Development of microsatellite markers followed a modified enrichment protocol by Ostrander et al. (1992) described in Maak et al. (2003). A genomic DNA library was established from 10 female Red Mason bees (*Osmia rufa* L.). Recombinants were screened with their respective microsatellite probes CA/GT, GA/CT or GAA/TCC. Positive clones were sequenced with the ALFexpress AutoRead sequencing kit according to the manufacturer and runs were performed on an ALFexpress II DNA analysis system (Amersham Pharmacia Biotech). Primers were designed using OLIGO 5.1 (MedProbe). PCR amplification of polymorphic loci was done in 25 µL reactions (puRE Taq Ready-To-Go system, Amersham Biosciences) containing 50–100 ng genomic DNA, 12.5 pmol of each fluorescently labeled forward and unlabelled reverse primer. After an initial denaturation step of 180 s at 94 °C, the amplification proceeded for 30–35 cycles as follows; 60 s at 94 °C, 60 s annealing at primer specific temperatures (Tab. I) and 120 s at 72 °C (Thermocycler UNO II, Biometra). Alleles were separated on an ABI 377 DNA Analyzer and fragment size was determined with GeneScan software and Gene scan 500 [Tamra] length standards (Applied Biosystems).

2.2. Population comparisons

For population comparisons we analyzed female bees from 10 European *O. rufa* populations collected in Germany (Halle - HALL, Schkopau - SCHK, Leau - LEAU, Oranienbaum - ORAN, Gatersleben - GATE, Tübingen - TUEB), The Netherlands (Hilvarenbeek - HILV), Italy (Castel di Decima - ROME), Hungary (Kescemet - KESC) and Cyprus (Foini - FOIN). Five of the German populations (HALL, SCHK, LEAU, ORAN, GATE) came from the same province (for further details see Tab. II). Populations representing two subspecies of the close sister species *Osmia cornuta* (*O. c. cornuta* – Halle/Germany, *O. c. neoregaena* – Foini/Cyprus) served as reference populations. Samples were collected from artificial trap nests (only one female per nest) or by random netting around natural breeding

Table I. Details of 6 microsatellite loci developed from the Red Mason bee (*Osmia rufa*). Repeat motifs refer to the dominant satellite sequence.

Locus	Primers (5'–3')	Repeat motif	Annealing temp. (°C)	cloned allel (bp)	EMBL acc. nr.
Oru10	TTTCATGTTCCGTATTGTCA TGTTCGCTTCCAAAATCA	$(AC)_{11}$	50	158	AJ884679
OruS4	GAACGAAACACCACTGTCTT CACGGCGAGACGAAT	$(AC)_{10}$	50	197	AJ884680
OruE5	CGGAGACTTGGTTGAAAAT AAGCACTACCACCTTTCTTTA	$(GA)_{13}$	50	99	AJ884681
OruS8	TTGGAAAAGAAGCGGATGAG CACCCTCGGAACCACTCTC	$(AG)_{14}$	51	118	AJ884682
OruC4	CGTAGAAAACGAACCCTGTAA CGATAGCCGTATGGTAGCAC	$(CT)_{13}$	52	197	AJ884683
OruA8	TCGCGATGTATCGTGTTCCTT GGCTGGCGGCTGTCTAAG	$(GAA)_9$	54	165	AJ884684

Table II. Mean effective (A_E) and observed (A_O) allele number, expected (H_E) and observed (H_O) heterozygosity of 10 *O. rufa* populations and 2 subspecies of *O. cornuta*. The HWE column contains the outcome of the test for significance.

Population	Geographic Location	N	A_O / A_E ±S.E.	H_O / H_E ±S.E.	HWE *P*-value
HALL	51°28'42" N, 11°58'08" E	25	4.000 ± 0.633 / 2.350 ± 0.368	0.527 ± 0.052 / 0.523 ± 0.068	n.s.
SCHK	51°23'57" N, 11°58'13" E	20	4.333 ± 0.333 / 2.084 ± 0.355	0.450 ± 0.098 / 0.447 ± 0.090	n.s.
LEAU	51°44'02" N, 11°48'05" E	20	5.000 ± 0.516 / 2.420 ± 0.412	0.525 ± 0.036 / 0.540 ± 0.058	n.s.
ORAN	51°47'53" N, 12°24'02" E	29	5.000 ± 0.516 / 2.435 ± 0.360	0.506 ± 0.088 / 0.554 ± 0.049	n.s.
GATE	51°49'11" N, 11°17'18" E	27	4.000 ± 0.258 / 2.287 ± 0.293	0.482 ± 0.082 / 0.514 ± 0.079	n.s.
TUEB	48°31'38" N, 09°03'43" E	20	4.667 ± 0.422 / 2.557 ± 0.319	0.525 ± 0.042 / 0.581 ± 0.047	n.s.
HILV	52°13'55" N, 05°11'01" E	34	4.500 ± 0.342 / 2.289 ± 0.270	0.495 ± 0.053 / 0.526 ± 0.064	n.s.
ROME	41°45'01" N, 12°25'55" E	24	3.833 ± 0.477 / 2.021 ± 0.257	0.347 ± 0.061 / 0.466 ± 0.064	n.s.
KESC	46°53'35" N, 19°41'34" E	9	3.667 ± 0.422 / 2.305 ± 0.301	0.574 ± 0.097 / 0.520 ± 0.074	n.s.
FOIN	34°53'53" N, 32°50'14" E	16	2.833 ± 0.654 / 1.967 ± 0.335	0.469 ± 0.152 / 0.387 ± 0.125	n.s.
O. c. neor.	34°53'53" N, 32°50'14" E	4	2.500 ± 0.428 / 1.868 ± 0.259	0.464 ± 0.120 / 0.406 ± 0.091	-
O. c. corn.	51°28'42" N, 11°58'08" E	6	1.667 ± 0.494 / 1.276 ± 0.184	0.056 ± 0.035 / 0.148 ± 0.095	-

sites. To test for further cross species applicability of primers we additionally incorporated individuals of *Osmia lignaria propinqua* (Utah/USA, N = 3) and *Osmia fulviventris* (Rome/Italy, N = 3).

Genetic diversity measures such as observed allele number (A_O), observed (H_O) and expected heterozygosity (H_E) (Nei, 1973) were computed in GENEPOP version 3.4 (Raymond and Rousset, 1995). The effective alle number (A_E) was estimated in GenAlEx V5.1 (Peakall and Smouse, 2001). GENEPOP was used for calculating linkage disequilibrium, Hardy-Weinberg expectations (HWE) and tests of heterozygote deficiencies per locus and population. HWE tests were performed per locus using a Markov chain method (Guo and Thompson, 1992). Probability values were corrected with the sequential Bonferroni method when multiple tests were performed. A gene tree based on chord distances (Cavalli-Sforza and Edwards, 1967) was constructed in POPULATIONS (Olivier Langella, CNRS UPR9034) and drawn in TREEVIEW (Page, 1996). A Bayesian approach was taken to cluster

populations at group level using the population mixture analysis option in BAPS 3.0 (Corander et al., 2003, 2004). The program applies a stochastic optimization instead of a Markov Chain Monte Carlo (MCMC) algorithm to infer population structuring. Kullback-Leibler (KL) distances (Anderson and Thompson, 2002; Corander et al., 2003) were calculated to quantify divergence between clusters. Additionally, we applied a principle coordinate analysis (PCA) to a F_{ST} distance (FSTAT; Goudet, 1995) to describe the geographical pattern of *O. rufa* populations. The results were visualized in a 3D scatter plot. A Mantel test (Mantel, 1967) was used to infer a possible correlation between geographical and genetic distance (Mantel Nonparametric Test Calculator, V. 2.00; written by A. Liedloff).

3. RESULTS

3.1. Microsatellite isolation and characterization

A total of 940 recombinants were isolated of which 43 appeared positive after hybridization (4.6%). Only 21 loci harbored more than four repeat units with an averaged length of 10 repeats. After PCR testing of 15 loci only six microsatellites could be selected for further analyses (Tab. I).

3.2. Population tests

Degrees of polymorphism in *Osmia rufa* populations were moderate (Tab. II). Observed allele numbers per loci measured as follows: Oru10 (8), OruS4 (6), OruE5 (8), OruS8 (10), OruC4 (6), OruA8 (7). All *O. rufa* populations proved polymorphic for the six selected loci except FOIN (66.7%). Mean A_E and mean expected H_E measured 1.967 ± 0.335 (FOIN) – 2.557 ± 0.319 (TUEB) and 0.387 ± 0.125 (FOIN) – 0.581 ± 0.047 (TUEB), respectively. No deviations from HWE expectations were detected. Significant heterozygote deficiency was only observed for locus Oru10 in ROME.

Chord distances proved rather small among continental European populations (*Dc* = 0.187–0.337) resulting in a gene tree with largely insignificant bootstrap support values (Fig. 1). Only the island population FOIN from Cyprus was clearly separated (*Dc* = 0.492–0.657). High genetic homogeneity among European populations except FOIN was also confirmed by Bayesian structure analysis. All continental

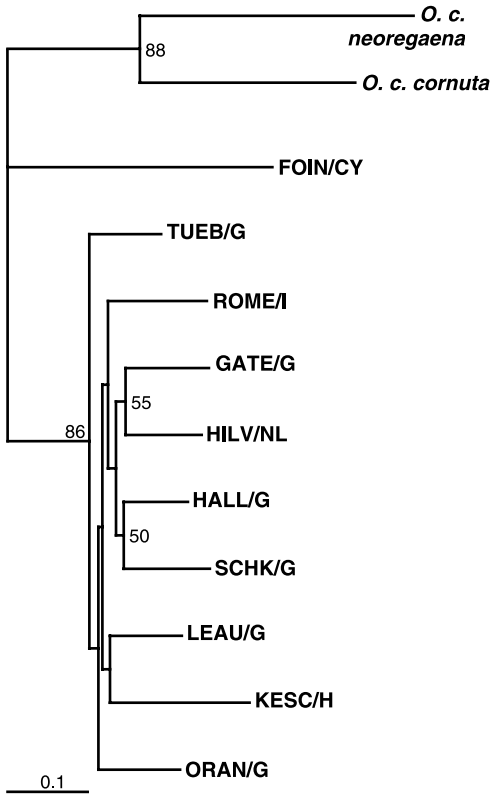

Figure 1. Rooted neighbour-joining (NJ) tree of 10 *Osmia rufa* populations based on chord distances (*Dc*). Numbers refer to bootstrap support values (only values > 50% are presented). Abbreviations are as follows: Germany (G) – Halle (HALL), Schkopau (SCHK), Leau (LEAU), Oranienbaum (ORAN), Gatersleben (GATE), Tübingen (TUEB); The Netherlands (NL) – Hilvarenbeek (HILV); Italy (I) – Castel di Decima (ROME); Hungary (H) – Kescemet (KESC) and Cyprus (CY) – Foini (FOIN).

European populations assigned to a single group whereas FOIN formed a group of its own. KL-distances between the two groups measured 2.495. F_{ST} measures among continental *O. rufa* populations range from 0.009 (HALL/SCHK) to 0.113 (SCHK/ROME) (Fig. 2). The Mantel test revealed a significant correlation between geographic distance and genetic distance (Z = 1947.3, r = 0.49, $P < 0.025$).

3.3. Other species

Osmia cornuta neoregaena and *Osmia cornuta cornuta* appeared less polymorphic than

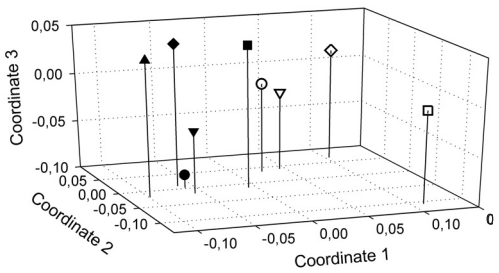

Figure 2. Principle coordinate analysis of F_{ST} distances for 9 continental *Osmia rufa* populations. Solid symbols refer to populations from Saxony-Anhalt (Germany): Circle – GATE, triangle down – ORAN, square – LEAU, diamond – HALL, triangle up – SCHK. Open symbols indicate other European *O. rufa* populations: Circle – TUEB (Germany), triangle down – HILV (The Netherlands), square – KESC (Hungary), diamond – ROME (Italy). The first three coordinates explain 71.64% of the total variation.

O. rufa. All microsatellites except OruS4 harbored more than one allele in *O. c. neoregaena* whereas three loci OruS4, OruE5 and OruC4 were monomorphic in *O. c. cornuta*. Alleles found at locus OruE5 in *O. c. neoregaena* exhibited distinct allele length variants, alternative to the predominant dinucleotide variation. Tests of HWE were not carried out considering the low number of individuals tested. All applied distance measures significantly differentiated *O. c. neoregaena* and *O. c. cornuta* from *O. rufa* (e.g. *Dc* = 0.644–0.808, KL-distance = 4.120–5.074). Most microsatellites amplified polymorphic alleles in two further *Osmia* species: *O. lignaria propinqua* (OruS4: $A_O = 3$, OruS8: $A_O = 2$, OruC4: $A_O = 2$) and *O. fulviventris* (Oru10: $A_O = 2$, OruE5: $A_O = 3$, OruS8: $A_O = 3$, OruC4: $A_O = 2$).

4. DISCUSSION

Number and length of polymorphic microsatellites isolated from *Osmia rufa* are similar to other bee studies (Green et al., 2001; Beveridge and Simmons, 2004). Only 50% of microsatellites consisted of loci with more than 10 perfect repeat units, a finding that corroborates marker reports from other Hymenopteran species (Estoup et al., 1993; Green et al., 2001). Since longer repeat stretches were obtained from a hamster (Cricetinae, Mammalia) library generated in parallel (Neumann and Jansman, 2004), an extreme selection bias towards shorter microsatellites through the PCR enrichment procedure itself can be largely excluded. However, there are few reports describing the cloning of a much higher portion of large microsatellite alleles e.g. in *Andrena* and *Lasioglossum* (e.g. Mohra et al., 2000; Kukuk et al., 2002; Paxton et al., 2003). Whether this is a taxon or isolation procedure specific phenomenon remains to become inquired. Noteworthy is the amplification of polymorphic alleles in a further three *Osmia* species suggesting a much wider usage of our microsatellites.

Allelic polymorphism and heterozygosity in *O. rufa* are comparable to the genetic diversity found in social Hymenopteran species such as *Apis mellifera* (Estoup et al., 1995) or *Bombus pascuorum* (Widmer and Schmid-Hempel, 1999). *O. rufa* populations from Germany, The Netherlands, Italy and Hungary appear genetically very homogeneous. The obtained population pattern is strikingly similar to that of other European bee species even so only data from social bees are available so far (Estoup et al., 1996; Widmer and Schmid-Hempel, 1999). There is no significant geographical structuring among continental *O. rufa* populations. Even bees from Italy do not appear as genetically distinct as it might be expected from their transalpine location. In contrast, Widmer and Schmid-Hempel (1999) showed a clear separation between *B. pascuorum* populations from north and south of the Alps. Genetic distance approaches clearly separate the *O. rufa* population from Cyprus (FOIN) from all other investigated populations. Significant differences between island and continental populations have also been found in other species such as European bumble bees (Estoup et al., 1996; Widmer et al., 1998). Founder events and interrupted gene flow are mainly responsible for the genetic distinctiveness of island populations. In that respect, it is not surprising that the FOIN sample exhibited the lowest levels of genetic polymorphisms and even proved homozygous at two loci. That strongly argues for a drift effect in a small founding population.

Low F_{ST} values and a significant isolation-by-distance effect account for gene flow among continental *Osmia* populations. A correlation

between geographical location and genetic distance was also found in a solitary bee species from North America (Danforth et al., 2003) but not in a European bumble bee (Widmer and Schmid-Hempel, 1999). Long distance migration is one possible mechanism which may contribute to the low genetic separation of European *Osmia* populations. *O. rufa* females are philopatric and gene flow may therefore largely to be attributed to male migratory behavior prior to female emergence (e.g. Torchio and Tepedino, 1980). Unfortunately, the proportions of migrating males and females as well as their typical migration distances are not yet known for the species.

At this stage we cannot decide whether the lack of spatial genetic structure and the low genetic variability in *O. rufa* are the result of slow genomic evolution or a signal for a recent population expansion. Extensive gene flow alone is not likely to account for the observed pattern because it does not explain low overall genetic variability. Large scale homoplasy effects can reduce resolution of potential population differences (Viard et al., 1998). However, they do not lead to the relatively short size of microsatellites found in our study.

The observed genetic homogeneity among European *O. rufa* populations could be the consequence of a post glacial range extension in Europe as proposed for *B. pascuorum* (Widmer and Schmid-Hempel, 1999). There is no doubt that the last glacial maximum influenced the spatial structure of European insect populations (see also Cooper et al., 1995). However, the apparent high proportion of small microsatellites found in almost all studies on Hymenoptera strongly implies a more general phenomenon such as low genomic evolution. One major reason for that could be the relatively low effective population size in Hymenopteran species as a result of their haplo-diploid sex-determination system (reviewed by Packer and Owen, 2001). Other likely causes are the high population turnover within a mosaic metapopulation system which leads to repeated founder events and the monandric mating system. These mechanisms impede the spread of novel mutations and perhaps the growth of microsatellite loci. Sociality or non-sociality may not significantly influence genetic variability in Hymenopterans because solitary bees exhibit a similar degree of genetic variability as social species (see also Danforth et al., 2003)

Although the isolation of microsatellites in Hymenopterans may still provide a considerable task, it is necessary to apply a much larger number of loci to explore the population structures of wild European bees in more detail. The apparent low genomic evolution in bees clearly masks the effects of potential mechanisms involved in population differentiation. As a consequence, many conclusions drawn from studies based on small marker sets, including the presented one, can only provide a starting point for the understanding of the population genetics in bees.

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Résumé – Des microsatellites pour analyser les structures de populations chez l'Abeille maçonne *Osmia rufa* (Hymenoptera, Megachilidae). Les abeilles sauvages solitaires jouent un rôle important dans les écosystèmes terrestres en tant que pollinisateurs, pourtant elles sont de plus en plus menacées dans les régions de cultures. Pour développer des stratégies de protection et de gestion il est nécessaire de disposer d'informations concernant la variabilité génétique et la structure des populations, données qui manquaient jusqu'à présent. Nous avons isolé des microsatellites polymorphes chez l'Abeille maçonne rousse, *Osmia rufa cornigera* (Rossi) afin d'étudier la diversité génétique et la structure des populations d'une abeille sauvage représentative.
Une bibliothèque génomique partielle a été enrichie en microsatellites (CA/GT, GA/CT, GAA/TCC) et criblée avec les sondes correspondantes. Sur 940 recombinants 43 clones sont apparus positifs après hybridation. Seuls 21 locus présentaient plus de 4 répétitions de motifs et six d'entre eux ont été choisis pour analyser la population (Tab. I). Pour comparer les populations nous avons analysé des

femelles provenant de neuf populations européennes continentales (dont six d'Allemagne) et d'une population insulaire (Chypre). L'espèce voisine *O. cornuta* Latr. a servi de référence.

Les degrés de polymorphisme dans les populations d'*O. rufa* sont modérés (Tab. II). On n'a pas noté d'écarts par rapport à l'équilibre de Hardy-Weinberg. Les distances génétiques entre les populations continentales sont faibles et il n'y a pas de groupement géographique net (Fig. 1). Seule la population de Chypre est bien séparée. L'homogénéité génétique relative des populations européennes, à l'exception de Chypre, a été confirmée par l'analyse statistique bayésienne et l'analyse en coordonnées principales (Fig. 2). Le test de Mantel a montré une corrélation significative entre la distance géographique et la distance génétique, ce qui indique un flux de gènes entre les populations continentales. Cet échange de gènes peut s'expliquer par des migrations de mâles sur de longues distances. *O. c. cornuta* et *O. c. neoregaena* présentent moins de variabilité que *O. rufa*. Chez *O. fulviventris* et *O. lignaria propinqua* aussi on a trouvé des allèles polymorphes pour la plupart des microsatellites.

Le nombre et la faible longueur relative des microsatellites que nous avons trouvés chez *O. rufa* concordent avec les résultats obtenus chez d'autres espèces d'abeilles. Le nombre d'allèles et l'hétérozygotie sont comparables à la diversité génétique des Hyménoptères sociaux. L'uniformité relative des populations d' *O. rufa* en Europe est vraisemblablement due à la perte de polymorphismes par le système haplo-diploïde de détermination du sexe et par de fortes fluctuations de populations. Un plus grand nombre de marqueurs est nécessaire pour apprécier l'influence des autres processus de population tels que le flux de gènes ou le repeuplement post-glaciaire.

***Osmia rufa* / Megachilidae / structure de la population / diversité génétique / microsatellite**

Zusammenfassung – Mikrosatelliten zur Analyse von Populations-Strukturen bei der Roten Mauerbiene (*Osmia rufa*). Solitäre Wildbienen spielen als Bestäuber eine wichtige Rolle in terrestrischen Ökosystemen, sind jedoch in anthropogenen Kulturlandschaften zunehmend gefährdet. Zur Entwicklung von Schutz- und Management-Strategien sind Informationen zur genetischen Variabilität und Populationsstruktur notwendig, die bislang nicht vorliegen. Wir isolierten polymorphe Mikrosatelliten bei der Roten Mauerbiene (*Osmia rufa conringera*), um die genetische Diversität und Populations-Struktur einer repräsentativen Wildbienenart untersuchen zu können.

Eine partielle genomische Bibliothek wurde mit Mikrosatelliten (CA/GT, GA/CT, GAA/TCC) angereichert und mit den entsprechenden Sonden gescreent. Aus 940 Rekombinanten wurden 43 positive Klone isoliert. Nur 21 Loci wiesen mehr als 4 Motiv-Wiederholungen auf, von denen 6 für die Populations-Analysen ausgewählt wurden (Tab. I). Für Populationsvergleiche standen 9 europäische Festlandspopulationen (davon 5 aus Sachsen-Anhalt, Deutschland) und eine Insel-Population aus Zypern zur Verfügung. Als Referenz diente die Schwesterart *O. cornuta.*

Der Polymorphie-Grad in *O. rufa*-Populationen erwies sich als moderat (Tab. II). Abweichungen vom Hardy-Weinberg-Gleichgewicht wurden nicht beobachtet. Die genetischen Distanzen zwischen den Festlands-Populationen von *O. rufa* sind eher klein und es ergaben sich keine deutlichen geografischen Cluster (Abb. 1). Nur die Population aus Zypern ist klar abgegrenzt. Die relative genetische Homogenität der Europäischen Populationen mit Ausnahme von Zypern wurde durch Bayesische Statistik und eine Hauptkoordinaten-Analyse (Abb. 2) bestätigt. Ein Mantel-Test zeigte eine signifikante Korrelation von geographischer und genetischer Distanz, welche auf Genfluss zwischen den kontinentalen Populationen hindeutet. Dieser Genaustausch zwischen den *O. rufa*-Populationen könnte durch weiträumige Migrationen der Männchen erklärt werden. *O. c. cornuta* und *O. c. neoregaena* sind weniger variabel als *O. rufa*. Für die meisten Mikrosatelliten wurden auch bei *O. fulviventris* und *O. lignaria propinqua* polymorphe Allele gefunden. Die Anzahl und relative Kürze der von uns bei *O. rufa* gefundenen Mikrosatelliten entsprechen den Ergebnissen bei anderen Bienenarten. Allelzahl und Heterozygotie sind vergleichbar mit der genetischen Diversität sozialer Hymenopteren. Die relative Uniformität von *O. rufa*-Populationen in Europa geht wahrscheinlich auf den Verlust von Polymorphismen durch das haplo-diploide Paarungssystem und starke Populationsfluktuationen zurück. Um den Einfluss von anderen Populationsprozessen wie Genfluss und postglaziale Wiederbesiedlung zu beurteilen, ist eine größere Anzahl von Mikrosatellitenmarkern erforderlich.

***Osmia rufa* / Megachilidae / Mikrosatellit / genetische Variabilität / Populations-Struktur**

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