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PRIMER NOTE

Nine polymorphic microsatellite loci for the parasitic wasp *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae)

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Abstract

Nine polymorphic microsatellite loci were isolated from *Neotypus melanocephalus* (Gmelin), a parasitoid of the parasitic large blue butterfly *Maculinea nausithous*. Allelic diversity and heterozygosity were quantified in samples from the Upper Rhine valley in Southwest Germany.

Keywords: contemporary gene flow, *Maculinea*, microsatellite, *Neotypus*, population genetics

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Neotypus melanocephalus (Gmelin 1790, Hymenoptera: Ichneumonidae) is a solitary endoparasitoid of the predatory large blue *Maculinea nausithous* (Lepidoptera: Lycaenidae). Butterflies of the genus *Maculinea* have an extraordinary life history as the parasites of *Myrmica* ants (Thomas & Settele 2004). After feeding for 2–3 weeks inside the flower heads of specific host plants, larvae leave the flower heads and are adopted by workers of their host ant. During the short period of phytophagy, some *M. nausithous* larvae are attacked by *N. melanocephalus* inside the flower heads of the host plant *Sanguisorba officinalis*. In such instances, the parasitoid follows its butterfly host by developing inside the ants' nest.

Most species live in patchy environments. In order to persist they must be able to disperse between populations and colonize empty habitat patches. Dispersal is usually measured by either marking individuals directly or indirectly by measuring gene flow. Highly polymorphic genetic markers like microsatellites in conjunction with new statistical methods can be a powerful tool for the analysis of contemporary gene flow (e.g. Berry *et al*. 2004).

For the DNA extraction, 20 adult wasps excluding heads and wings were used. Samples were homogenized in 350 μL cetyltrimethyl ammonium bromide (CTAB) buffer (Doyle & Doyle 1990) and digested with 20 μL proteinase K for 60 min. Three hundred and fifty microlitres chloroform/isoamylalcohol (1:24) was added, shaken for 2 min and spun at 13 000 r.p.m. ×g for 10 min. The supernatant was precipitated using 900 μL of freezing cold ethanol and stored for 12 h at –20 °C. After centrifugation for 25 min at 13 000 r.p.m. ×g, the pellet was washed three times with 100 μL 95% ethanol. The dry DNA was dissolved in pure water. An enriched library was made by ECOGENICS GmbH from size-selected genomic DNA ligated into SAULA/SAULB-linker (5′-GCGGTACCCGGGAAGCTTGG/5′-GATCCCAAGCTTCCCGGGTACCGC) (Armour *et al*. 1994) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschie *et al*. 2000a, b). Of 192 recombinant colonies screened, 116 gave positive signals after hybridization. Plasmids from 73 positive clones were sequenced and analysed using the program BIODEDIT (URL: http://www.mbio.ncsu.edu/BioEdit/). The software PRIMER 3 was used to design 18 oligonucleotide primers (Rozen & Skaletsky 2000). One primer from each pair was end-labelled with fluorescent dyes (FAM, JOE, TAMRA).

Allelic variability was tested with *N. melanocephalus* larvae from one site close to Landau in southwest Germany. For the DNA extraction, the very small-sized larvae were incubated for 10 min at 95 °C in 100 μL 5% Chelex-100 (10 mM Tris, pH 7.5). Before using the DNA, the tissue was spun. For the polymerase chain reaction (PCR), 2 μL of the supernatant was used in 8 μL reaction volumes containing 2 pmol of each forward and reverse primer and 4 μL multiplex PCR kit (QIAGEN Multiplex PCR Kit). Multiplex PCR was performed with loci Neo35 and Neo42 (label: FAM), Neo34 (JOE) and Neo39 (TAMRA) in the first reaction, Neo40 (FAM), Neo37 and Neo09 (JOE) in a second reaction, and Neo02 (FAM) and Neo10 (JOE) in a third reaction. All PCR amplifications were conducted with a Primus-96

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Table 1 Characteristics of the *Neotypus melanocephalus* microsatellite loci: repeat motif of the sequenced clone, size range of alleles, number of alleles, primer sequence, specific annealing temperature, observed and expected heterozygosities and GenBank Accession nos, with * indicating significant departures from Hardy–Weinberg equilibrium ($P < 0.001$). Genomic sequences were deposited to the European Bioinformatics Institute EMBL-BMI, Cambridge

Locus	Repeat	Size (bp)	No. of alleles (no. of individuals)	Primer sequence (5′–3′)	T_a (°C)	H_O	H_E	Accession no.
Neo02	$(GA)_{23}$	144–168	6 (32)	F: GTCCTGCATTCGACGACAC R: TTCGCTCTTCTTCGTCCACT	60	0.156*	0.588	AM117941
Neo09	$(CA)_{22}$	348–410	8 (32)	F: CCACTCTCGGCTACGGATTA R: TCTGTACGGTATGGCGTCTG	60	0.267*	0.860	AM117942
Neo10	$(GT)_{22}$	163–223	5 (30)	F: TCTGTACGGTATGGCGTCTG R: GTAGCGGGTATCGGAGGAAC	60	0.344*	0.620	AM117943
Neo34	$(GT)_{14}$	158–186	5 (33)	F: ACAGTGTCCTCCCCCTTTTC R: CCATCTCCCTAGCACTCTCG	60	0.576	0.647	AM117944
Neo35	$(GT)_{12}$	311–319	4 (32)	F: GGTGGAGTTCAAGGGTCGTA R: CCCCCTAGCCATCAATTTTA	60	0.563	0.662	AM117945
Neo37	$(GT)_{12}$	309–311	2 (31)	F: CGAAGCAGCGAGGAAGTTTA R: TCTCAACGTGTGCGAGTTTC	61	0.226	0.337	AM117946
Neo39	$(CT)_{22}$	186–224	5 (33)	F: GCTTCGTCGTTCGCTTTTT R: TTACACGGGGCAATTAGAGC	61	0.515	0.662	AM117947
Neo40	$(CA)_{16}$	136–150	5 (32)	F: TACTCCCTCTCGGACTGCTC R: AATTTCGGAACAATGCAAATG	60	0.406*	0.776	AM117948
Neo42	$(GT)_{41}$	203–243	10 (33)	F: GGTTCGTCCCATCGCTACTA R: CGAAGTGACGCAATTCTCCT	60	0.515*	0.831	AM117949

thermocycler (MWG) using a denaturation step at 95 °C for 15 min, followed by 35 cycles of 30 s at 94 °C, 60 s at 57 °C, 1 min 30 s at 72 °C, and then a final extension step of 30 min at 60 °C. Reactions were held at 4 °C before separation. Fragments were separated on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with internal size standard GeneScan-500 ROX (Applied Biosystems). Because of the haplodiploid nature of inheritance of Hymenoptera such as *N. melanocephalus*, only the data for 33 females were used to determine the allelic diversity and to calculate the observed number of heterozygotes. Individuals that were homozygous across all loci were considered as male larvae and excluded from the analysis. Nine loci were polymorphic with the allelic richness ranging from two to 10, and their observed heterozygosity ranging from 0.16 to 0.58 (Table 1). Expected heterozygosity was calculated using ARLEQUIN (Schneider *et al*. 2000). For four of the loci, the observed heterozygosity was significantly lower than the expected heterozygosity (Table 1). The deviations from the Hardy–Weinberg equilibrium might be affected by the presence of null alleles. In the loci Neo02, Neo09, Neo10, Neo35, Neo37 and Neo40, four PCR amplifications of DNA did not succeed in 1, 3, 1, 1, 2 and 1 individuals, respectively. The linkage disequilibrium was tested with GENEPOP (Rayment & Rousset 1995). Significant linkage disequilibria ($P < 0.05$, Fisher's exact test) were found with the locus pairs Neo34 and Neo35, Neo02 and Neo37, Neo10 and Neo40, Neo37 and Neo42, and Neo39 and Neo42.

The nine microsatellite markers described will be used for the analysis of population genetic structure of the highly endangered parasitoid *N. melanocephalus*. Because immigration and emigration are key population parameters, genotypic analyses could give valuable information about the intensity of contemporary gene flow in *N. melanocephalus* metapopulations.

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