

University of Groningen

## Characterisation and cross-amplification of polymorphic microsatellite loci in ant-associated root-aphids

Ivens, A. B. F.; Kronauer, D. J. C.; Boomsma, J. J.

*Published in:*  
Conservation genetics resources

*DOI:*  
[10.1007/s12686-010-9293-3](https://doi.org/10.1007/s12686-010-9293-3)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2011

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Ivens, A. B. F., Kronauer, D. J. C., & Boomsma, J. J. (2011). Characterisation and cross-amplification of polymorphic microsatellite loci in ant-associated root-aphids. *Conservation genetics resources*, 3(1), 73-77. <https://doi.org/10.1007/s12686-010-9293-3>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

## Characterisation and cross-amplification of polymorphic microsatellite loci in ant-associated root-aphids

A. B. F. Ivens · D. J. C. Kronauer ·  
J. J. Boomsma

Received: 28 June 2010 / Accepted: 20 July 2010 / Published online: 1 August 2010  
© The Author(s) 2010. This article is published with open access at Springerlink.com

**Abstract** Twenty-six polymorphic microsatellite loci were developed for four species of ant-associated root-aphids: *Geoica utricularia*, *Forda marginata*, *Tetraneura ulmi* and *Anoecia corni*. We found up to 9 alleles per locus, with an average of 4.8. We also report polymorphic cross-amplification of eleven of these markers between different pairs of study species. Furthermore, we tested previously published aphid microsatellites and found one locus developed for *Pemphigus bursarius* to be polymorphic in *G. utricularia*. These microsatellite markers will be useful to study the population structure of aphids associated with the ant *Lasius flavus* and possibly other ants. Such studies are relevant because: 1. *L. flavus* mounds and their associated flora and fauna are often key components in protected temperate grasslands, and 2. *L. flavus* and its diverse community of root-aphids provide an interesting model system for studying the long-term stability of mutualistic interactions.

**Keywords** Microsatellites · Root-aphids · Mutualism · Aphidoidea (Hemiptera) · Pemphigidae · Anoeciidae

---

A. B. F. Ivens (✉)  
Theoretical Biology Group, Centre for Ecological and  
Evolutionary Studies, University of Groningen,  
P.O. Box 11103, 9700 CC Groningen, The Netherlands  
e-mail: A.B.F.Ivens@rug.nl

A. B. F. Ivens · D. J. C. Kronauer · J. J. Boomsma  
Centre for Social Evolution, Department of Biology,  
University of Copenhagen, Universitetsparken 15,  
2100 Copenhagen, Denmark

D. J. C. Kronauer  
Museum of Comparative Zoology, Harvard University,  
26 Oxford Street, Cambridge, MA 02138, USA

Mutualistic interactions between species are widespread and play key roles in ecosystem stability and diversity (Stachowicz 2001; Bastolla et al. 2009). In Northwest Europe, the yellow meadow ant *Lasius flavus* keeps up to fourteen species of mutualistic root-aphids in its nests (Pontin 1978; Heie 1980; Godske 1991). The ants actively tend the aphids, which provide them with honeydew (Pontin 1978). The nest mounds are markers of high grassland biodiversity and long-term habitat stability (Dean et al. 1997; Blomqvist et al. 2000; Lenoir 2009). However, despite the decline of European temperate grasslands in recent decades and the associated losses in plant and invertebrate biodiversity (WallisDeVries et al. 2002), neither the sociobiology of the ants (but see Boomsma et al. 1993), nor the biology of the root-aphids (Pontin 1978; Godske 1991, 1992) have been extensively studied. To facilitate molecular ecological approaches in the study of this mutualism, we developed DNA microsatellite markers for the four commonest species: *Forda marginata*, *Tetraneura ulmi*, *Geoica utricularia* and *Anoecia corni*.

Samples for genomic library construction for *Forda marginata*, *Tetraneura ulmi*, and *Anoecia corni* were collected in 2007 from an ant-nest on the Dutch island of Schiermonnikoog (53°29'03.5"N; 6°13'46.1"E) whereas *Geoica utricularia* was collected near Dejret, Denmark (56°12'54.2"N; 10°24'48.2"E). All samples for molecular analysis were preserved in 96% ethanol.

Genomic DNA was extracted using the QIAGEN DNeasy Blood & Tissue kit and enriched for poly-CA and poly-CT microsatellite containing fragments using the protocol by Rütten et al. (2001). We designed PCR primers for the flanking regions of repetitive motifs using the web-based software Primer 3 (Rozen et al. 2000).

Primers were tested on Schiermonnikoog samples collected in 2007, 2008 and 2009 and on samples collected

**Table 1** Characteristics of 26 polymorphic microsatellite loci in different species of ant-associated root-aphids

| Locus | Species                   | Primer sequence (5′–3′)<br>(F: forward, R: reverse)           | Repeat motif  | Size range (bp) | N   | N <sub>a</sub> | H <sub>E</sub> | H <sub>O</sub> | T <sub>a</sub> (°C) | Nr. of cycles x | Primer concentration (μM) | Multiplex mix | Genbank accession number |
|-------|---------------------------|---|---|-----------------|-----|----------------|----------------|----------------|---------------------|-----------------|---------------------------|---------------|--------------------------|
| Gu1   | <i>Geoica utricularia</i> | F: ATCAAACGAACGAACCCGAAT<br>R: GCGAAAAGTTATGGCGTTTGT          | (GT) <sub>8</sub>   | 113–118         | 5   | 4              | 0.740          | 1.000          | 50                  | 40              | 0.35                      | Gu-3          | HMS82813                 |
| Gu2   | <i>Geoica utricularia</i> | F: CGCGATTAGATCTCGGAATG<br>R: AAATCGTATAAAAAGTAAAGGCGTTAT     | (GT) <sub>11</sub>  | 158–177         | 227 | 5              | 0.613          | 0.361          | 50                  | 40              | 0.15                      | Gu-2          | HMS82814                 |
| Gu3   | <i>Geoica utricularia</i> | F: TATCGTGGGACACAGACAT<br>R: CGGGCTATACCGCATACACT             | (TA) <sub>9</sub>   | 192–208         | 169 | 7              | 0.665          | 1.000          | 50                  | 40              | 0.15                      | Gu-1          | HMS82815                 |
| Gu4   | <i>Geoica utricularia</i> | F: CTGCTGCTCGTCCGACTTA<br>R: GCAGATAAAAAACTGTAGCCTTGA         | (TG) <sub>6</sub> C (AT) <sub>12</sub>                                    | 206–222         | 8   | 4              | 0.602          | 0.125          | 50                  | 35              | 0.35                      | Gu-3          | HMS82816                 |
| Gu5   | <i>Geoica utricularia</i> | F: CACAGGACGGTAACTTAATATAG<br>R: ACACTTTTTCGGCAATTTTCGT       | (GT) <sub>15</sub>  | 164–214         | 214 | 6              | 0.569          | 0.145          | 50                  | 40              | 0.15                      | Gu-2          | HMS82817                 |
| Gu6   | <i>Geoica utricularia</i> | F: ATCAAACGGTCTGGCATGTA<br>R: CAATATCTCATCTGCCAGCAA           | (TG) <sub>3</sub> CG (GT) <sub>8</sub>                                    | 151–200         | 199 | 7              | 0.539          | 0.337          | 50                  | 40              | 0.15                      | Gu-2          | HMS82818                 |
| Gu7   | <i>Geoica utricularia</i> | F: GTTAAAGGAACTTTACGCTCTACG<br>R: CATATAAATAAAAAACGTCCTGTAGGC | (CA) <sub>3</sub> TA (CA) <sub>5</sub>                                    | 87–103          | 13  | 4              | 0.698          | 0.000          | 50                  | 40              | 0.35                      | Gu-3          | HMS82819                 |
| Gu8   | <i>Geoica utricularia</i> | F: TATACAGTCCGGCAGATA<br>R: GTTCGTTGCTCGTGCACCTT              | (AC) <sub>10</sub>  | 233–237         | 199 | 3              | 0.479          | 0.060          | 50                  | 40              | 0.15                      | Gu-1          | HMS82820                 |
| Gu9   | <i>Geoica utricularia</i> | F: CGCGGTTATGAAAATGTA<br>R: CTCGCTGTGTGACACCTT                | (CA) <sub>13</sub>  | 223–250         | 184 | 8              | 0.800          | 0.799          | 50                  | 40              | 0.15                      | Gu-1          | HMS82821                 |
| Gu10  | <i>Geoica utricularia</i> | F: CGCGCTAAAGAAAGTTTCA<br>R: TTACGTTAAACA(AC)ACGAGGATTTAT     | (GT) <sub>19</sub>  | 228–261         | 14  | 8              | 0.763          | 0.786          | 50                  | 40              | 0.35                      |               | HMS82822                 |
| Gu11  | <i>Geoica utricularia</i> | F: CGGTTACCCGTAAAAGGCTTA<br>R: AAAICGCAATGACAGTCACG           | (CA) <sub>11</sub>  | 145–153         | 223 | 6              | 0.729          | 0.677          | 50                  | 40              | 0.15                      | Gu-2          | HMS82823                 |
| Gu12  | <i>Geoica utricularia</i> | F: GAGCCAACTGCCCGTTATAG<br>R: CGGTTTTATTTAAGGTCTCGAA          | (GT) <sub>12</sub> GC (GT) <sub>25</sub><br>A (GT) <sub>4</sub>           | 106–138         | 10  | 3              | 0.460          | 0.000          | 60                  | 45              | 0.15                      |               | HMS82824                 |
| Gu13  | <i>Geoica utricularia</i> | F: TCGCCGTCGACTATTTTACA<br>R: AGTTACGTCGGGAGAAAT              | (CAG) <sub>7</sub> (N) <sub>21</sub> (TC) <sub>10</sub>                   | 202–218         | 188 | 7              | 0.754          | 1.000          | 50                  | 40              | 0.15                      | Gu-1          | HMS82825                 |
| Gu15  | <i>Geoica utricularia</i> | F: TTTTACGGGCTAAACCCTATTT<br>R: CCAATACGGATCCCAACTTTT         | (GA) <sub>15</sub> (A) <sub>4</sub> (GA) <sub>3</sub><br>(A) <sub>9</sub> | 165–167         | 10  | 2              | 0.180          | 0.200          | 50                  | 40              | 0.25                      |               | HMS82826                 |
| Fm1   | <i>Forda marginata</i>    | F: CCTCCAATTACCGTTCAACC<br>R: GAAGAACGTGACACGGGATA            | (TG) <sub>22</sub> CG (TG) <sub>5</sub>                                   | 182–259         | 154 | 9              | 0.458          | 0.253          | 53                  | 37              | 0.15                      |               | HMS82827                 |
| Fm3   | <i>Forda marginata</i>    | F: TCTGATTTTTCGTTCTGCCA<br>R: CGCGGCTCGTTACCTATTTA            | (AT) <sub>10</sub>  | 225–349         | 138 | 6              | 0.494          | 0.246          | 50                  | 40              | 0.15                      |               | HMS82828                 |
| Fm4   | <i>Forda marginata</i>    | F: CATTACGTGTGAGTGAATATAGTTTT<br>R: TGGTTTAAACGACGGATTTTC     | (AC) <sub>14</sub>  | 178–200         | 162 | 7              | 0.465          | 0.167          | 50                  | 35              | 0.15                      |               | HMS82829                 |

**Table 1** continued

| Locus | Species                | Primer sequence (5'–3')<br>(F: forward, R: reverse)      | Repeat motif  | Size range (bp) | N   | N <sub>a</sub> | H <sub>E</sub> | H <sub>O</sub> | T <sub>a</sub> (°C) | Nr. of cycles x | Primer concentration (μM) | Multiplex mix | Genbank accession number |
|-------|------------------------|--|---|-----------------|-----|----------------|----------------|----------------|---------------------|-----------------|---------------------------|---------------|--------------------------|
| Fm6   | <i>Forda marginata</i> | F: TCACTCGCCTAGCGTTATTC<br>R: GTGGCCGTAGCATGTCACTA       | (T) <sub>11</sub> ATGA (T) <sub>23</sub>  | 250–280         | 125 | 4              | 0.709          | 0.920          | 50                  | 45              | 0.15                      |               | HM582830                 |
| Tu1   | <i>Tetranевра ulmi</i> | F: CGGGTGGGTGGGTACATTAT<br>R: ATACGTTGAGCCAACTACCG       | (GT) <sub>4</sub> GAT(AG) <sub>5</sub> T-<br>(GA) <sub>10</sub> (A) <sub>6</sub> (N) <sub>6</sub> (T) <sub>17</sub> | 218–241         | 89  | 2              | 0.164          | 0.000          | 50                  | 35              | 0.25                      |               | HM582831                 |
| Tu2   | <i>Tetranевра ulmi</i> | F: TCCGACATACGTTTAAACCAAAA<br>R: ATGACACCCCTGCCCACTATC   | (TA) <sub>7</sub> (TG) <sub>8</sub>   | 157–159         | 60  | 2              | 0.180          | 0.000          | 50                  | 40              | 0.25                      | Tu-1          | HM582832                 |
| Tu3   | <i>Tetranевра ulmi</i> | F: CGCCGTAATAATAATAACAACAA<br>R: CACGAGACCAAGAGATAAGGAAA | (A) <sub>11</sub> (AT) <sub>6</sub> (TA) <sub>2</sub><br>(C) <sub>3</sub> (GT) <sub>9</sub>                         | 234–264         | 89  | 5              | 0.702          | 0.921          | 50                  | 35              | 0.25                      |               | HM582833                 |
| Tu4   | <i>Tetranевра ulmi</i> | F: TTATTGCAACCCACACCTTG<br>R: ACGCGACGGATAGAAAATACG      | (GT) <sub>26</sub> G (GT) <sub>3</sub>  | 182–203         | 94  | 6              | 0.636          | 0.904          | 50                  | 40              | 0.25                      | Tu-1          | HM582834                 |
| Tu10  | <i>Tetranевра ulmi</i> | F: AGTATACGGGTCTCGCCAAC<br>R: GGAGCAAGTCCGATCGTTAT       | (TAA) <sub>3</sub> TGA (TAA) <sub>7</sub>   | 233–248         | 87  | 3              | 0.226          | 0.253          | 50                  | 40              | 0.25                      |               | HM582835                 |
| Tu11  | <i>Tetranевра ulmi</i> | F: CGGAGAACGCGTATTGATTT<br>R: CGTGCGCGTGTCAAAAAGTAT      | (GT) <sub>9</sub> (TA) <sub>5</sub>   | 194–200         | 89  | 4              | 0.396          | 0.393          | 50                  | 35              | 0.25                      |               | HM582836                 |
| Ac6   | <i>Anoecia corni</i>   | F: CGAGGCATATTCAAAATGTAAGA<br>R: CAGCATTAACACGAAATGCAA   | (AT) <sub>3</sub> G (TA) <sub>9</sub><br>C (AT) <sub>2</sub>  | 148–164         | 6   | 2              | –              | –              | 45                  | 45              | 0.25                      |               | HM582837                 |
| Ac8   | <i>Anoecia corni</i>   | F: AATAATAATTCGTGGCGTTGC<br>R: CGCCGTAGAAAGCAAATAATATC   | (ATT) <sub>10</sub>   | 160             | 4   | 1              | –              | –              | 45                  | 45              | 0.25                      |               | HM582838                 |

N number of tested samples, N<sub>a</sub> number of alleles, H<sub>E</sub> expected heterozygosity, H<sub>O</sub> observed heterozygosity, T<sub>a</sub> annealing temperature

near Dejret in 2007 (*Anoecia* spp.). DNA for microsatellite screening was extracted using 200  $\mu$ l 20%-Chelex<sup>®</sup> 100 resin (Fluka) (Walsh et al. 1991). PCR-cocktails had a total volume of 10  $\mu$ l, consisting of 0.8 mM dNTPs, 2 mM MgCl<sub>2</sub>, 1 $\times$  PCR buffer, 0.25 U AmpliTaq Gold<sup>®</sup> DNA Polymerase (Applied Biosystems), 1  $\mu$ l of DNA template and a varying concentration of primers (Table 1). Several primer pairs were multiplexed in PCR (Table 1). The amplification conditions were 95°C for 5 min,  $x$  number of cycles of 95°C for 30 s,  $T_a$  for 30 s and 72°C for 30 s (1 min for Gu3, Gu8, Gu9, Gu10 and Gu13) and a final extension of 15 min at 72°C. The respective  $x$  and  $T_a$  for each primer are listed in Tables 1 and 2.

Amplified fluorescent labeled PCR-products were run on an ABI-PRISM 3130XL (Applied Biosystems) sequencer and chromatograms were analyzed in Genemapper (Applied Biosystems). Expected and observed heterozygosities and deviations from Hardy–Weinberg Equilibrium (HWE) were determined using GENALEX 6.2 (Peakall and Smouse 2006). Occurrence of Linkage Disequilibrium (LD) was assessed using Genepop 4.0 (Rousset 2008).

The fourteen markers developed for *Geoica utricularia* were tested on 5–227 aphids. All markers were polymorphic, with 5.3 alleles per locus on average (Table 1). The four polymorphic markers for *Forda marginata* were tested together with three cross-amplifying markers (Gu6, Gu11, Gu13) on 125–162 aphids yielding 6.0 alleles on average (Tables 1 and 2). The six microsatellite markers for *Tetraneura ulmi* had 3.7 alleles on average in 60–94 tested aphids (Table 1). Observed and expected heterozygosities

are given in Tables 1 and 2. Since all species reproduce asexually, deviations from HWE and presence of LD are expected (Ivens et al., in preparation). All loci indeed showed significant deviation from HWE, except for Gu15 in *Geoica utricularia*, Fm4 and Gu11 in *Forda formicaria*, and Tu10 in *Tetraneura ulmi*. In *G. utricularia* the majority of the loci pairs (65%) had significant LD, with most pairs not in LD involving Gu1 and Gu15. All pairs of *T. ulmi* were in LD, except for Tu10–Tu2, Tu10–Tu1, Tu2–Tu11 and Tu1–Tu11. In *F. marginata*, all loci pairs were in LD.

The two primer pairs developed for the genus *Anoecia* amplified across *Anoecia* species but were not extensively tested. We merely report these loci here for future reference.

Cross-amplification was tested for all markers except Gu12 and Fm5 (Table 2), yielding eleven markers that amplified in one or more additional species. Moreover, most markers used (species specific and cross-amplified) for *Forda marginata* were also suitable for the sibling species *Forda formicaria*. The loci Fm3, Fm4, Fm6 and Gu13 proved to be diagnostic for distinguishing between *F. marginata* and *F. formicaria* (Table 2). Three markers from *Pemphigus bursarius* (Pb02 (Miller et al. 2000)) and *P. spyrothecae* (97PS12 and 98PS8 (Johnson et al. 2000)) were tested for cross-amplification in our focal species, but only Pb02 reliably cross-amplified in *Geoica utricularia* (Table 2).

Although we enriched specifically for (CA)<sub>n</sub> and (CT)<sub>n</sub> repeats, the aphid DNA appeared to be especially AT-rich, including repeats that were suitable for microsatellite

**Table 2** Cross-amplifications of microsatellite markers in different species of ant-associated root-aphids

| Locus             | Cross-amplified species                   | Size range (bp) | $N$ | $N_a$ | $H_E$ | $H_O$ | $T_a$ (°C) | Nr. of cycles $x$ | Primer concentration ( $\mu$ M) | Genbank accession number |
|-------------------|---|-----------------|-----|-------|-------|-------|------------|-------------------|---------------------------------|--------------------------|
| Gu6               | <i>Forda marginata</i>                    | 151–176         | 159 | 5     | 0.681 | 0.672 | 49         | 40                | 0.15                            | HM582818                 |
| Gu11              | <i>Forda marginata</i>                    | 135–147         | 162 | 6     | 0.489 | 0.234 | 49         | 40                | 0.15                            | HM582823                 |
| Gu13              | <i>Forda marginata</i>                    | 143–178         | 159 | 5     | 0.430 | 0.000 | 45         | 45                | 0.15                            | HM582825                 |
| Tu11              | <i>Forda marginata</i>                    | –               | 2   | –     | –     | –     | 49         | 40                | 0.15                            | HM582836                 |
| Fm3               | <i>Forda formicaria</i>                   | 121             | 18  | 1     | 0.000 | 0.000 | 50         | 40                | 0.15                            | HM582828                 |
| Fm4               | <i>Forda formicaria</i>                   | 174–178         | 18  | 3     | 0.495 | 0.777 | 50         | 35                | 0.15                            | HM582829                 |
| Fm6               | <i>Forda formicaria</i>                   | 206–291         | 18  | 2     | 0.500 | 1.000 | 50         | 45                | 0.15                            | HM582830                 |
| Gu6               | <i>Forda formicaria</i>                   | 151–152         | 17  | 2     | 0.110 | 0.000 | 49         | 40                | 0.15                            | HM582818                 |
| Gu11              | <i>Forda formicaria</i>                   | 142–146         | 18  | 3     | 0.439 | 0.277 | 49         | 40                | 0.15                            | HM582823                 |
| Gu13              | <i>Forda formicaria</i>                   | 156             | 19  | 1     | 0.000 | 0.000 | 45         | 45                | 0.15                            | HM582825                 |
| Fm1               | <i>Anoecia corni</i> , <i>A. zirnitsi</i> | 110–134         | 7   | 3     | –     | –     | 45         | 45                | 0.25                            | HM582827                 |
| Tu2               | <i>Anoecia corni</i> , <i>A. zirnitsi</i> | 137–148         | 3   | 2     | –     | –     | 45         | 45                | 0.25                            | HM582832                 |
| Tu11              | <i>Anoecia corni</i> , <i>A. zirnitsi</i> | 69–126          | 7   | 5     | –     | –     | 45         | 45                | 0.25                            | HM582836                 |
| Ac 8              | <i>Anoecia zirnitsi</i> , <i>A. major</i> | 130–146         | 2   | 2     | –     | –     | 45         | 45                | 0.25                            | HM582838                 |
| Pb02 <sup>a</sup> | <i>Geoica utricularia</i>                 | 118–124         | 8   | 2     | –     | –     | 50         | 40                | 0.20                            | AF267192                 |

$N$  number of tested samples,  $N_a$  number of alleles,  $H_E$  expected heterozygosity,  $H_O$  observed heterozygosity,  $T_a$  annealing temperature

<sup>a</sup> Developed by Miller et al. 2000 for the lettuce root-aphid *Pemphigus bursarius*

design. This observation is in accordance with earlier findings (Weng et al. 2007).

In conclusion, the 26 newly developed microsatellite markers presented here cover a large proportion of the known root-aphid fauna associated with *L. flavus* and other ant species (Heie 1980), and will be useful for detailed studies of the ecology and evolution of this mutualistic association.

**Acknowledgments** This study was supported by a PhD-Grant from the Centre for Ecological and Evolutionary Studies, University of Groningen and grants from the Pieter Langerhuizen Fund and the Nicolaas Mulerius Foundation, awarded to A.B.F.I., as well as a grant from the Danish National Research Foundation to J.J.B. that funded D.J.C.K.'s postdoctoral work in Copenhagen. The authors would like to thank Sylvia Mathiasen, Thijs Janzen and Elzemies Geuverink for help in the laboratory and in the field, Ole E. Heie and Maurice Jansen for help with aphid taxonomy and Franz J. Weissing and Ido Pen for discussion.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

- Bastolla U, Fortuna MA, Pascual-Garcia A, Ferrera A, Luque B, Bascompte J (2009) The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature* 458(7241):1018–1091. doi:10.1038/nature07950
- Blomqvist MM, Olff H, Blaauw MB, Bongers T, Van der Putten WH (2000) Interactions between above- and belowground biota: importance for small-scale vegetation mosaics in a grassland ecosystem. *Oikos* 90(3):582–598
- Boomsma JJ, Wright PJ, Brouwer AH (1993) Social structure in the ant *Lasius flavus*—multi-queen nests or multi-nest mounds. *Ecol Entomol* 18(1):47–53
- Dean WRJ, Milton SJ, Klotz S (1997) The role of ant nest-mounds in maintaining small-scale patchiness in dry grasslands in central Germany. *Biodivers Conserv* 6(9):1293–1307
- Godske L (1991) Aphids in nests of *Lasius flavus* F. in Denmark I: faunistic description. *Entomol Meddr* 59(3):85–89
- Godske L (1992) Aphids in nests of *Lasius flavus* F. in Denmark II: population dynamics. *Entomol Meddr* 60(1):21–26
- Heie OE (1980) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I. Fauna *Entomol Scand* 9:1–236
- Johnson PCD, Llewellyn KS, Amos W (2000) Microsatellite loci for studying clonal mixing, population structure and inbreeding in a social aphid, *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). *Mol Ecol* 9(9):1445–1446
- Lenoir L (2009) Effects of ants on plant diversity in semi-natural grasslands. *Arthropod Plant Interact* 3(3):163–172. doi:10.1007/s11829-009-9066-7
- Miller NJ, Birley AJ, Tatchell GM (2000) Polymorphic microsatellite loci from the lettuce root aphid, *Pemphigus bursarius*. *Mol Ecol* 9(11):1951–1952
- Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6(1):288–295. doi:10.1111/j.1471-8286.2005.01155.x
- Pontin AJ (1978) Numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). *Ecol Entomol* 3(3):203–207
- Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Res* 8:103–106
- Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics methods and protocols: methods in molecular biology*. Humana Press, Totowa, NJ, pp 365–386
- Rütten K, Schulz I, Olek K, Uhl G (2001) Polymorphic microsatellite markers in the spider *Pholcus phalangioides* isolated from a library enriched for CA repeats. *Mol Ecol Notes* 1(4):255–257
- Stachowicz JJ (2001) Mutualism, facilitation, and the structure of ecological communities. *Bioscience* 51(3):235–246
- WallisDeVries MF, Poschlod P, Willems JH (2002) Challenges for the conservation of calcareous grasslands in Northwestern Europe: integrating the requirements of flora and fauna. *Biol Conserv* 104(3):265–273
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10(4):506–513
- Weng Y, Azhaguvel P, Michels GJ, Rudd JC (2007) Cross-species transferability of microsatellite markers from six aphid (Hemiptera: Aphididae) species and their use for evaluating biotypic diversity in two cereal aphids. *Insect Mol Biol* 16(5):613–622. doi:10.1111/j.1365-2583.2007.00757.x