Reproduction and dispersal in an ant-associated root aphid community – Supplementary material

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The dependence of the ant *Lasius flavus* on root aphids

The honeydew secreted by the aphids is the main source of carbohydrates for the subterranean *L. flavus* ants, as other subterranean carbon sources are limited in the salt marsh and chalk-grassland habitats of these ants. Stable isotope analyses have confirmed that in the presence of aphids there is a demonstrable intermediate trophic level between the vegetation and the ants, consistent with the aphids being an intermediate foodweb node (A.B.F. Ivens & M. Schrama, unpublished data). *L. flavus* ants have also been recorded to eat a large proportion of their root aphids, suggesting that they also utilize their “dairy cattle” to increase protein acquisition (Pontin 1958; 1961; 1978; Ivens *et al.* in press). This implies that, similar to other subterranean ants, *L. flavus* depends on root aphid husbandry for producing colonies large enough to reproduce (Way 1963; Hölldobler & Wilson 1990).

Tables and Figures

Table S1 Results of tests for the presence of Linkage Disequilibrium for *ramet* and *genet* datasets of *Geoica utricularia, Tetraneura ulmi* and *Forda marginata.* G-tests for each available pair of loci were run using the Markov Chain algorithm of Raymond and Rousset (1995). P-values statistically significant at a 5%-level are given in bold, indicating pairs of loci with significant Linkage Disequilibrium. The parameter setting for dememorisation was 10000, the number of batches was set at 20, and the number of iterations per batch was 5000.

Ramet data			
Locus 1	**Locus 2**	**P-Value**	**S.E.**
Geoica utricularia			
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu2	Gu2	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu5	Gu5	**0.000**	0.000
Gu6	Gu6	**0.000**	0.000
Gu6	Gu6	**0.000**	0.000
Gu6	Gu6	**0.000**	0.000
Gu6	Gu6	**0.000**	0.000

Genet data			
Locus 1	**Locus 2**	**P-Value**	**S.E.**
Gu2	Gu3	**0.000**	0.000
Gu2	Gu5	**0.001**	0.001
Gu2	Gu6	**0.016**	0.003
Gu2	Gu8	**0.008**	0.002
Gu2	Gu9	0.483	0.011
Gu2	Gu11	0.078	0.005
Gu2	Gu13	0.054	0.005
Gu5	Gu3	**0.001**	0.000
Gu5	Gu6	**0.003**	0.001
Gu5	Gu8	**0.003**	0.001
Gu5	Gu9	0.904	0.006
Gu5	Gu11	**0.010**	0.003
Gu5	Gu13	**0.011**	0.003
Gu6	Gu3	**0.003**	0.002
Gu6	Gu8	**0.007**	0.002
Gu6	Gu9	1.000	0.000
Gu6	Gu11	0.124	0.010

Gu6	Gu6	**0.000**	0.000	Gu6	Gu13	0.057	0.007
Gu8	Gu8	**0.000**	0.000	Gu8	Gu3	**0.002**	0.001
Gu8	Gu8	**0.000**	0.000	Gu8	Gu9	0.235	0.010
Gu8	Gu8	**0.000**	0.000	Gu8	Gu13	**0.002**	0.001
Gu9	Gu9	**0.000**	0.000	Gu9	Gu3	0.903	0.007
Gu9	Gu9	**0.000**	0.000	Gu9	Gu13	0.097	0.009
Gu11	Gu11	**0.000**	0.000	Gu11	Gu3	**0.012**	0.003
Gu11	Gu11	**0.000**	0.000	Gu11	Gu8	**0.049**	0.004
Gu11	Gu11	**0.000**	0.000	Gu11	Gu9	0.338	0.013
Gu11	Gu11	**0.000**	0.000	Gu11	Gu13	**0.018**	0.003
Gu13	Gu13	**0.000**	0.000	Gu13	Gu3	**0.009**	0.002
Pairs with significant LD : 28				**Pairs with significant LD : 17**			
Tetraneura ulmi							
Tu1	Tu3	**0.000**	0.000	Tu1	Tu3	0.467	0.004
Tu1	Tu11	0.073	0.003	Tu1	Tu11	1.000	0.000
Tu2	Tu1	**0.000**	0.000	Tu2	Tu1	0.066	0.002
Tu2	Tu3	**0.000**	0.000	Tu2	Tu3	0.465	0.006
Tu2	Tu4	**0.000**	0.000	Tu2	Tu4	0.205	0.003
Tu2	Tu11	0.070	0.002	Tu2	Tu11	1.000	0.000
Tu4	Tu1	**0.000**	0.000	Tu4	Tu1	0.198	0.003
Tu4	Tu3	**0.000**	0.000	Tu4	Tu3	1.000	0.000
Tu4	Tu11	**0.000**	0.000	Tu4	Tu11	1.000	0.000
Tu10	Tu1	0.095	0.002	Tu10	Tu1	1.000	0.000
Tu10	Tu2	0.091	0.002	Tu10	Tu2	1.000	0.000
Tu10	Tu3	**0.000**	0.000	Tu10	Tu3	0.399	0.006
Tu10	Tu4	**0.000**	0.000	Tu10	Tu4	1.000	0.000
Tu10	Tu11	**0.000**	0.000	Tu10	Tu11	0.194	0.005
Tu11	Tu3	**0.000**	0.000	Tu11	Tu3	0.208	0.011
Pairs with significant LD : 11				**Pairs with significant LD : 0**			

Forda marginata							
Fm1	Fm3	**0.000**	0.000	Fm1	Fm3	**0.001**	0.001
Fm1	Fm4	**0.000**	0.000	Fm1	Fm4	**0.000**	0.000
Fm1	Fm6	**0.000**	0.000	Fm1	Fm6	**0.001**	0.001
Fm1	Gu6	**0.000**	0.000	Fm1	Gu6	0.870	0.006
Fm1	Gu11	**0.000**	0.000	Fm1	Gu11	**0.000**	0.000
Fm1	Gu13	**0.000**	0.000	Fm1	Gu13	**0.033**	0.006
Fm3	Fm4	**0.000**	0.000	Fm3	Fm4	**0.000**	0.000
Fm3	Fm6	**0.000**	0.000	Fm3	Fm6	**0.000**	0.000
Fm3	Gu6	**0.000**	0.000	Fm3	Gu6	0.060	0.005
Fm3	Gu11	**0.000**	0.000	Fm3	Gu11	**0.000**	0.000
Fm3	Gu13	**0.000**	0.000	Fm3	Gu13	**0.003**	0.001
Fm4	Fm6	**0.000**	0.000	Fm4	Fm6	**0.000**	0.000
Fm4	Gu6	**0.000**	0.000	Fm4	Gu6	0.537	0.009
Fm4	Gu11	**0.000**	0.000	Fm4	Gu11	**0.000**	0.000
Fm4	Gu13	**0.000**	0.000	Fm4	Gu13	**0.001**	0.001
Fm6	Gu6	**0.000**	0.000	Fm6	Gu6	0.167	0.006
Fm6	Gu11	**0.000**	0.000	Fm6	Gu11	**0.000**	0.000
Fm6	Gu13	**0.000**	0.000	Fm6	Gu13	**0.000**	0.000
Gu6	Gu11	**0.000**	0.000	Gu6	Gu11	**0.023**	0.003
Gu13	Gu6	**0.000**	0.000	Gu13	Gu6	0.107	0.004
Gu13	Gu11	**0.000**	0.000	Gu13	Gu11	**0.001**	0.000
Pairs with significant LD : 23				**Pairs with significant LD : 16**			

Figure S1 Neighbour-Joining trees for the three root aphid genera. Neighbour-Joining trees were constructed based on shared allele distances (DAS) (Jin & Chakraborty 1993). Genetic distances were calculated and trees were constructed in POPULATIONS 1.2.30 (Olivier Langella 1999) and displayed graphically in R 2.12.0 (R development core team 2010) using the plot.phylo() function in the ape package (Paradis *et al.* 2004). Genetic distance in the trees is given as the proportion of alleles that are not shared between multilocus genotypes (MLGs). Colours represent specific multilocus lineages (MLLs) consisting of several multilocus genotypes (MLGs) with names corresponding to those given in Table 2 and node numbers representing bootstrap values (10000 iterations). The *Forda* tree includes both *F. formicaria* (Ff- MLGs, all belonging to one MLL given in red) and *F. marginata* MLGs.

Figure S2 Spatial distribution of cumulative frequencies of Multilocus Genotypes (MLGs) of *Forda formicaria*. Distinct MLGs are represented by different hatching patterns. *n* gives the sample size for each transect location (location 1-8, with adjacent sample sites being 1 km apart – see Fig 1).

Figure S3 Spatial autocorrelation pattern for *Forda formicaria*. The plot shows genetic correlation coefficient *r* (solid line), its 95% confidence bars as determined by bootstrapping, and the 95% confidence interval around zero as expected from random distributions of genotypes (dashed lines), plotted across the seven distance classes (1-7 km). The result for the Mantel test of the correlation is provided in the left corner of the panel. Due to small sample sizes (see Figure S2), these tests remain inconclusive.

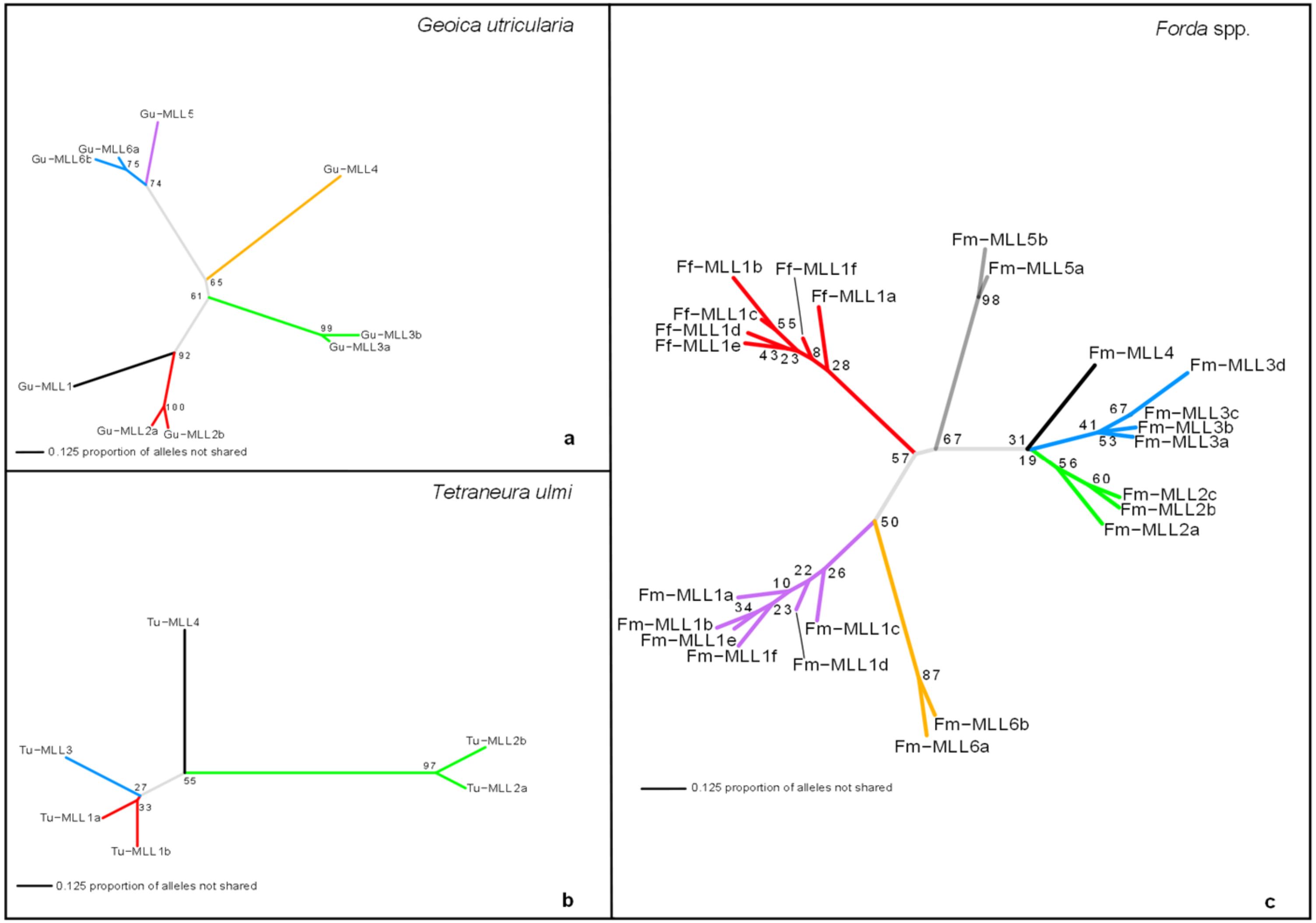
Geoica utricularia
Gu-MLL5
Gu-MLL6a
Gu-MLL6b
75
74
Gu-MLL4
65
61
99
Gu-MLL3b
Gu-MLL3a
92
Gu-MLL1
100
Gu-MLL2a
Gu-MLL2b
0.125 proportion of alleles not shared
a
Tetraneura ulmi
Tu-MLL4
Tu-MLL3
27
55
97
Tu-MLL2b
Tu-MLL2a
Tu-MLL1a
33
Tu-MLL1b
0.125 proportion of alleles not shared
b
Forda spp.
Fm-MLL5b
Fm-MLL5a
98
Ff-MLL1b
Ff-MLL1f
Ff-MLL1a
Ff-MLL1c
55
Ff-MLL1d
Ff-MLL1e
43
23
8
28
Fm-MLL4
Fm-MLL3d
67
Fm-MLL3c
41
Fm-MLL3b
53
Fm-MLL3a
67
31
57
19
56
60
Fm-MLL2c
Fm-MLL2b
Fm-MLL2a
50
22
26
10
Fm-MLL1a
34
23
Fm-MLL1b
Fm-MLL1e
Fm-MLL1f
Fm-MLL1c
Fm-MLL1d
87
Fm-MLL6b
Fm-MLL6a
0.125 proportion of alleles not shared
c

Figure S1

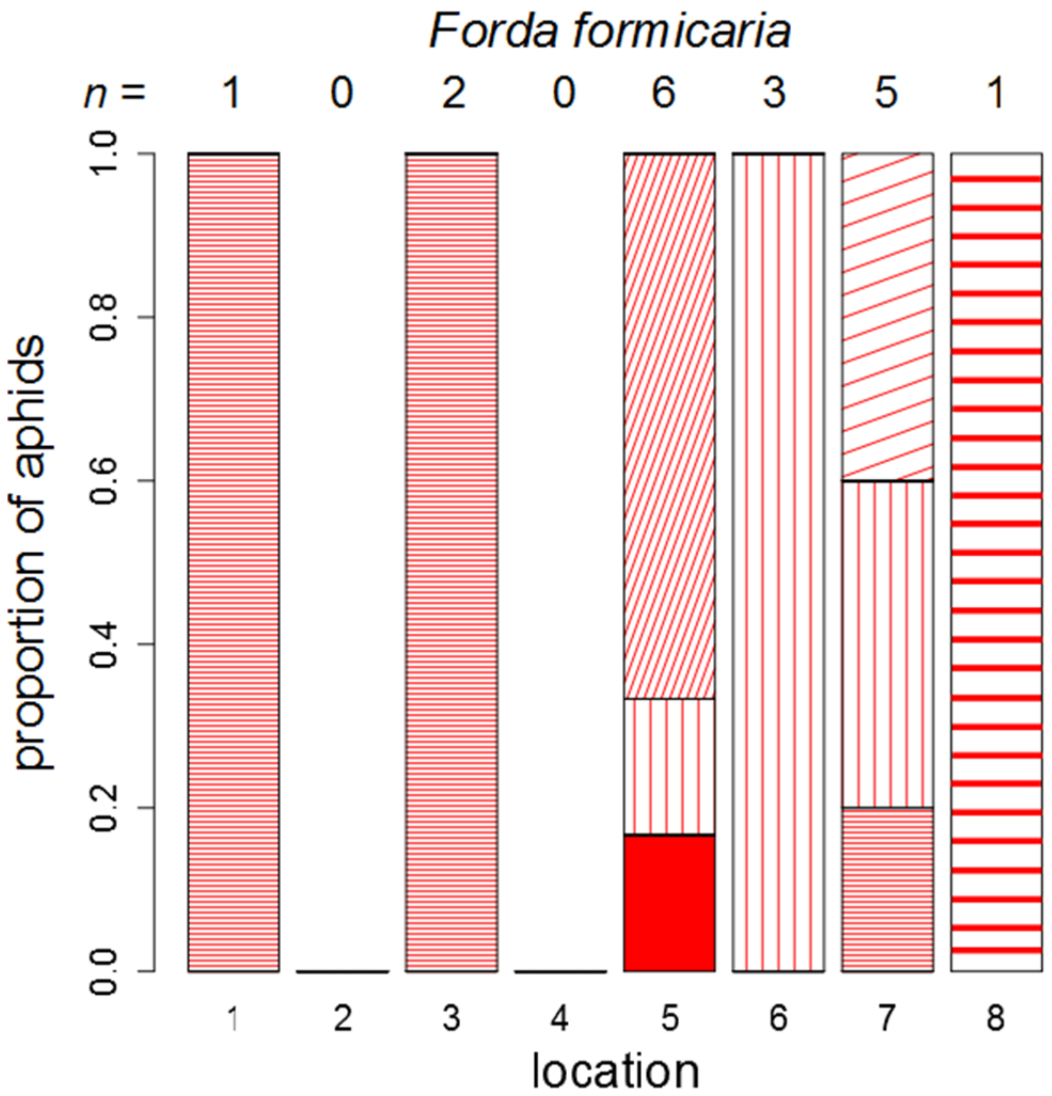
Forda formicaria
n =
1
0
2
0
6
3
5
1
proportion of aphids
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
8
location

Figure S2

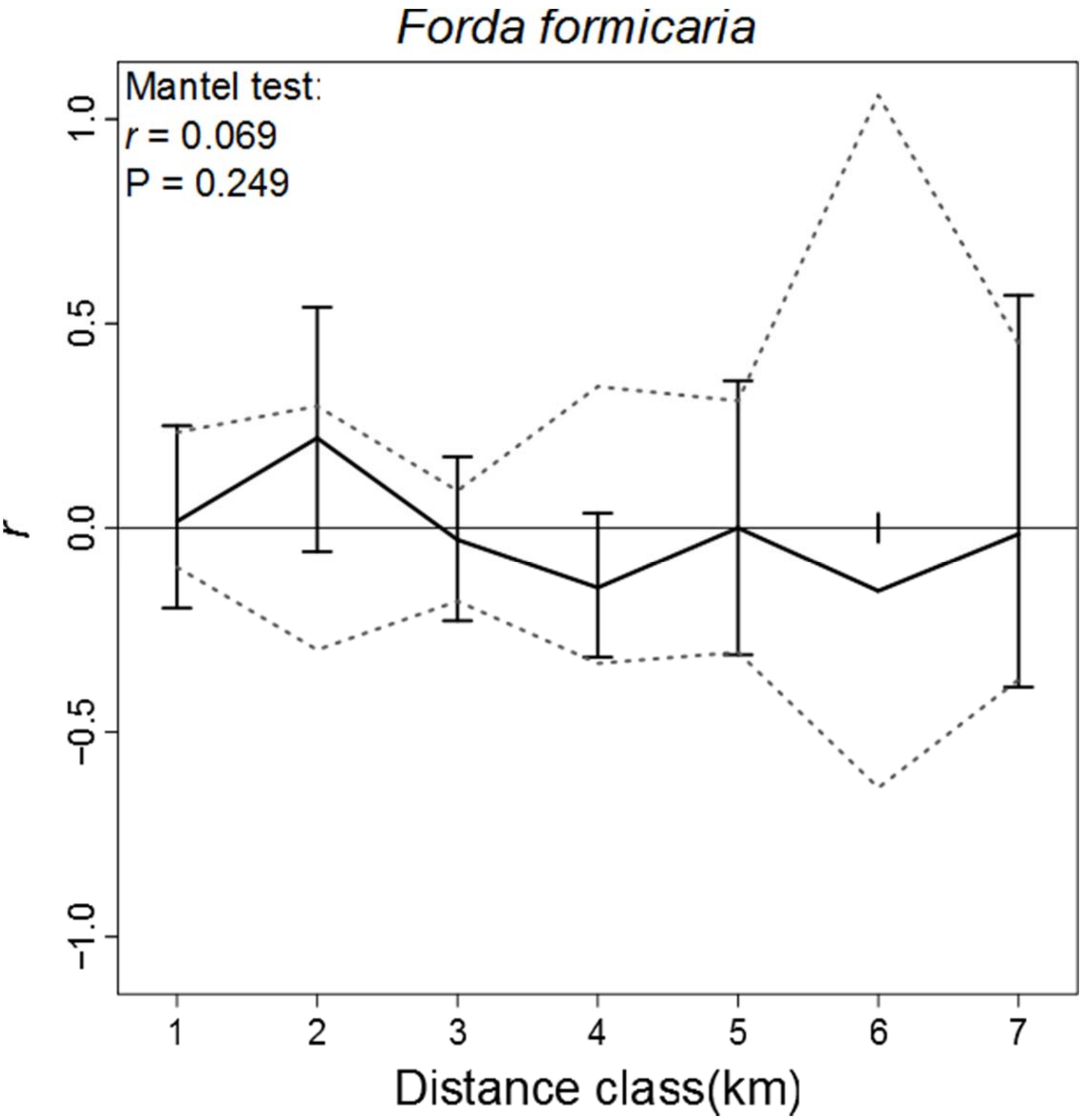
Forda formicaria
Mantel test:
r = 0.069
P = 0.249
r
1.0
0.5
0.0
−0.5
−1.0
1
2
3
4
5
6
7
Distance class(km)

Figure S3

References

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