Supporting Information

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Fig. S1. Population has a hybrid background. Morphometric analysis of (A) worker nest samples (n = 131) and (B) gyne individuals (n = 76) indicates that the study population (black dots) is intermediate between *F. aquilonia* (white rhombs) and *F. polyctena* (white squares). Discriminant vectors were calculated based on 16 and 25 quantitative morphological characters measured in workers and gynes respectively. The pessimistic leave-one-out cross-validation rejects only 3.8% and 1.3% of the classifications of workers and gynes, respectively, in the whole data set, with no rejections occurring in the study population.



Fig. 52. Distribution of mean pair-wise AFLP differences within and between the two genetic groups in males (*n* = 97). The values are calculated as means of each male to all other males in the same group or in the other group. The distributions of pair-wise differences within group W and between the groups do not overlap.



Fig. S3. Assignment of the females (gynes; n = 95) to the two male groups R and W. The assignment is based on 17 microsatellite loci.

Table S1.	Allele categories	in microsatellite,	AFLP, and a	allozyme mar	kers showing	the number
of alleles i	n each category a	nd mean numbe	r per indivio	lual		

	Total no.		Mean no. per individual			
Allele category	Loci	Alleles	W males	W females	R females	R males
Microsatellites						
Shared by all	16	24	11.5	23.0	22.8	11.3
Diagnostic						
R-specific						
With only heterozygous females	9	11	_	—	4.1	4.7
W-specific						
With only heterozygous females	1	1	0.3	0.3	_	_
With also homozygous females	7	7	3.0	3.9	_	—
Admixed						
Admixed from R to W	3	3	_	0.9	2.6	0.9
Admixed from W to R	5	5	2.0	5.0	3.0	_
Female-specific	3	3	0.0	0.8	1.2	—
Allozyme (Gpi)						
Admixed from W to R	1	1	0.8	0.5	0.2	—
AFLP						
Diagnostic						
R-specific, recessive		9	_	?	+	3.3
R-specific, dominant		4	_	_	+	1.8
W-specific, recessive		9	3.1	+	?	—
W-specific, dominant		3	1.3	+	_	—
Admixed						
Admixed from R to W, dominant		4	_	+	+	1.2
Admixed from W to R, dominant		1	0.8	+	+	
Female-specific		3	_	0.3	1.0	_

A dash indicates that no allele was observed; 0.0 indicates that alleles were observed but the number per individual was less than 0.05; + indicates that alleles were observed but the frequencies could not inferred because of dominance; ? indicates that the occurrence of the alleles could not be inferred because of dominance.

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Locus	W males	W females	W _{maternal}	R _{maternal}	R females	R males
fe13						
186	0.52	0.35	0.18	0.04	0.28	0.51
189	0.00	0.00	0.00	0.00*	0.22*	0.49*
198	0.48	0.65	0.82	0.96 ⁺	0.50 [†]	0.00
fe17						
110	0.18	0.42	0.67	0.56 [†]	0.28 ⁺	0.00
116	0.82	0.58	0.33	0.35	0.62	0.89
118	0.00	0.00	0.00	0.09*	0.10*	0.11*
fe19	0.00	0100	0.00	0.00	0110	
178	0 34*	0 24*	0 12*	0.00	0.00	0.00
184	0.34	0.24	0.58	0.00	0.50	0.00
194	0.32	0.40	0.00	0.00	0.30	0.20
100	0.52	0.17	0.00	0.00 0.22 [‡]	0.38 0.12 [‡]	0.00
fo 27	0.02	0.17	0.50	0.25	0.12	0.00
112	0.22	0.16	0.00	0.05	0.09	0.11
113	0.23	0.16	0.09	0.05	0.08	0.11
117	0.77	0.84	0.91	0.95	0.92	0.89
fe38						
71	0.55	0.38	0.20	0.32	0.46	0.60
73	0.10	0.48	0.80	0.35	0.32	0.29
75	0.35	0.14	0.00	0.33	0.22	0.11
fe42						
259	0.61	0.61	0.60	0.30	0.42	0.54
261	0.39	0.39	0.40	0.70	0.58	0.46
fe51						
90	1.00	1.00	1.00	1.00	0.79	0.51
92	0.00	0.00	0.00	0.00*	0.21*	0.49*
fe7						
56	0.00	0.13 [‡]	0.26 [‡]	0.51 [‡]	0.27 [‡]	0.00
66	0.20*	0.20*	0.20*	0.00	0.00	0.00
68	0.80	0.67	0.55	0.43 [†]	0.23 [†]	0.00
70	0.00	0.00	0.00	0.00*	0.08*	0.23*
72	0.00	0.00	0.00	0.00*	0.25*	0.50*
89	0.00	0.00	0.00	0.06*	0.25	0.26*
fl12	0.00	0.00	0.00	0.00	0.17	0.20
106	0.63	0.86	1 00	0 92	0.66	0.40
110	0.05	0.00	0.00	0.52	0.00	0.40
116	0.00	0.00	0.00	0.05	0.20	0.01
fico	0.57	0.14	0.00	0.05	0.00	0.09
1120	0.00	0.00	0.00	0.00*	0.20*	0 60*
111	0.00	0.00	0.00	0.00"	0.28"	0.00"
114	0.72	0.64	0.57	0.40	0.32	0.23
127	0.00	0.01	0.01	0.00	0.00	0.00
128	0.28	0.35	0.42	0.60	0.40	0.17
TIZI						
214	0.23	0.20	0.17	0.37	0.59	0.80
219	0.00	0.17'	0.34'	0.63	0.41	0.20
221	0.77*	0.63*	0.48*	0.00	0.00	0.00
fl29						
182	0.70	0.58	0.46	0.74	0.66	0.39
188	0.30	0.42	0.54	0.26 ⁺	0.16 ⁺	0.00
190	0.00	0.00	0.00	0.00*	0.18*	0.61*
fy10						
153	0.24*	0.14*	0.04*	0.00	0.00	0.00
157	0.76	0.86	0.96	1.00	1.00	1.00
fy12						
185	0.22	0.31	0.37	0.53	0.68	0.83
187	0.26	0.46	0.63	0.47	0.32	0.17
189	0.52*	0.24*	0.00*	0.00	0.00	0.00
fv13						
195	0.00	0.00	0.00	0.00*	0.22*	0.60*
107	0.03	0.11 [‡]	0.18 [‡]	0.00 [‡]	0.22	0.00
100	0.05	0.11	0.10	0.50	0.22	0.00
199	0.44	0.04	0.02	0.02	0.00	0.40
205	0.52^	0.25^	0.00^	0.00	0.00	0.00

Table S2. Microsatellite allele frequencies by genetic group and sex

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Table S2. Cont.

Locus	W males	W females	W $_{maternal}$	R $_{maternal}$	R females	R males
fy15						
221	0.40	0.42	0.42	0.00	0.08	0.18
222	0.00	0.07 [†]	0.14 [†]	0.43	0.50	0.50
232	0.29	0.38	0.44	0.57 ⁺	0.33 [†]	0.00
234	0.31*	0.13*	0.00*	0.00	0.00	0.00
240	0.00	0.00	0.00	0.00*	0.08*	0.32*
fy3						
185	0.54	0.57	0.60	0.36	0.58	0.08
188	0.46*	0.25*	0.03*	0.00	0.00	0.00
190	0.00	0.18 [†]	0.36 [†]	0.64	0.42	0.20

The columns $W_{maternal}$ and $R_{maternal}$ give the allele frequency estimates in the maternal component of the female genomes. The maternal frequencies $(x_{maternal})$ are estimated as $x_{maternal} = 2x_{female} - x_{male}$ and the frequencies for a locus are adjusted by setting estimates less than zero as 0. *Diagnostic alleles.

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⁺Admixed alleles shown in the group in which these alleles were admixed to. ^{*}Alleles specific (or almost specific) to females.

Marker	W males	W females	R females	R males
Microsatellit	es			
fe7 ⁵⁶	_	0.13	0.27	_
fe19 ¹⁹¹	0.02	0.17	0.12	_
fy13 ¹⁹⁷	0.03	0.11	0.22	_
AFLP				
1	_	0.11	0.38	_
2	—	0.12	0.24	—
3	—	0.08	0.18	—

Table S4.	Genetic distances	(D _A)	by	genetic	group	o and	sex
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D _A	W_{paternal}	$W_{maternal}$	R _{maternal}	R _{paterna}
W _{paternal}	—	0.16	0.25	0.39
W _{maternal}	0.02	_	0.08	0.38
R _{maternal}	0.02	0.01	_	0.34
R _{paternal}	0.02	0.01	0.01	—

Genetic distances (Nei D_{A} , above diagonal) and their SEs (below diagonal) among four haploid genomes (maternal R, maternal W, paternal R, paternal W) calculated on the basis of the allele frequencies in the suggested maternally and paternally inherited haploid genomes.

genotyp	b. ivior bes	tality estima	tes calculato	ed on t	the basis of missing
Group	Sex	l	Missing		Expected frequency

Group	Jex Missing		Expected frequency
R	Male	Alleles present in R females	0.89
W	Male	Alleles present in W females	0.37
R	Female	Homozygotes	0.65
W	Female	Homozygotes	0.16

As these genotypes were not observed, their expected frequencies estimate the fraction of individuals that have died. The markers do not cover all of the linkage groups, so the estimates are underestimates. *The expected frequencies for the missing genotypes are calculated on the basis of allele frequencies and represent a minimum estimate of mortality. Fraction of males that should have at least one of the alleles if they segregate at random is calculated from the allele frequencies in females as $P = 1 - \Pi(1 - x_{female})$. Fraction of females that should be homozygous for at least one of the alleles if they segregate at random is calculated from the expected homozygosities per locus as $P = 1 - \Pi(1 - HOM_{exp})$.

Table S6. Sample size and the number of loci studied for each marker type

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Sample	No. of Nests	No. of individuals	No. of AFLP loci	No. of microsatellite loci	No. of enzyme loci	No. of Mt gene*
Gynes						
R	2	25	81	17	1	1
W	4	70	81	17	1	1
Total	4	95				
Males						
R	2	35	81	17	1	1
W	4	62	81	17	1	1
Total	4	97				
Old Queens 2007						
R	1	12	_	14	_	_
W	1	11	_	14	_	_
Total	2	23				
Old Queens 2008						
R	1	10	_	9	_	_
W	6	40	_	9	_	_
Total	7	50				
Workers						
R	3	19	_	14	1	1
W	24	115	_	14	1	1
Total	24	134				

Dash indicates that the samples have not been studied with this marker.

*Only a subset of samples was used for Cytochrome b sequencing. These were two W gynes, five W males, one R gyne, four R males, five W workers, and three R workers.