Figure S1. Detailed genetic procedure for the first backcross session.
Dijon/w (Di/w) females were initially mated with Zimbabwe (Z30; green color) males. Resulting F1 ZW females (with colored eyes; magenta color) were mated with resulting F1 ZW males (white eyes; cyan color). Only resulting F2 ZW females with white eyes (cyan color) were backcrossed to Z30 males (Bc: F2 x F0). Then, a "sister x brother" cross was carried on the next generation (F3 x F3) between white-eyed flies. A backcross between F4 white-eyed females and Z30 males (F4 x F0), was followed by two successive "sister x brother" crosses (F5 x F5; F6 x F6) to yield sublines with homogeneous white-eyed progeny. Flies resulting of the F6 x F6 "sister x brother" cross produced white-eyed ZW-BC1 females and males which were tested in the "Analysis and Selection #1" session (see Figure 1).

Figure S2. Relationship between copulation status, fertility and fecundity in F0 strains.
Using the 9 possible crosses between the 3 F0 parental lines (Cs, Di/w, Z30; see Figure 2), we compared the fertility (ability to leave at least one adult progeny) and the fecundity (number of adult progeny per female) in females copulating within the two-hour observation period (2H) or during the following 24-hour period (>2H). The PCA reveals a high correlation between fertility and fecundity for each copulation status but not between copulation status. The amount of variation taken into consideration for each axis is indicated (F1: 42.26%; F2: 30.69%).

Figure S3. Fecundity in crosses between parental flies according to copulation status.
The number of adult progeny per female was counted in each of the cross involving a male (upper row under the bars) and a female (bottom row) according to the copulation status: mating within the two-hours observation period (COPULATION 2H) or during the 24 hours period (COPULATION >2H). Box-plots represent the 50% median data (the small horizontal bar indicates the median value, and the solid dot represents the mean; the whiskers represent the first and third quartiles). When the sample sizes are too small, single data points are shown as crosses. Different italic lowercase letters above whiskers indicate significant differences between means.

Figure S4. Variation of cuticular hydrocarbons ratio during the introgression procedure.

For each graph, the two dimensions axes represent the absolute amount of cuticular hydrocarbons (in ng). For the x-axis: 7, 11HD in females (left panels) and 7T (in males, right panels); For the y-axis 5,9HD and of 5T, respectively. The distribution for these two pairs of compounds (in females and in males) is shown for parental (F0) Z30 (green ellipse) and Di/w (cyan) flies. The dotted lines indicate the ratio between the two compounds for F0 flies together with three additional "intermediate" ratio values. The values shown for BC1, BC2 and BC3 (Analysis and Selection) sessions are shown according to the generation at which they were sampled. (See Figure 3).

Figure S5. Relationship between female and male cuticular hydrocarbons ratio.

(**A**) The distribution of the female (7,11HD/5,9HD) and male (7T/5T) ratio is shown on a two axis diagram according to the generation sampled during the introgression procedure. The position of the F0 Di/w line is highlighted by an arrow on the right upper corner. (**B**) Bars represent the level of the correlation between the 7T/5T ratio and the 7,11HD/5,9HD ratio at different generations of the introgression process. The significant correlations are indicated (*: $p<0.05$).

Figure S6. Correlation between cuticular hydrocarbons during the introgression procedure.

The level and sign of the correlation between pairs of CHs (indicated under the corresponding bars) are shown at different generations of the introgression procedure (the color code for generations is shown on the right side).

Figure S7. Correlation between copulation parameters and cuticular hydrocarbons ratio.

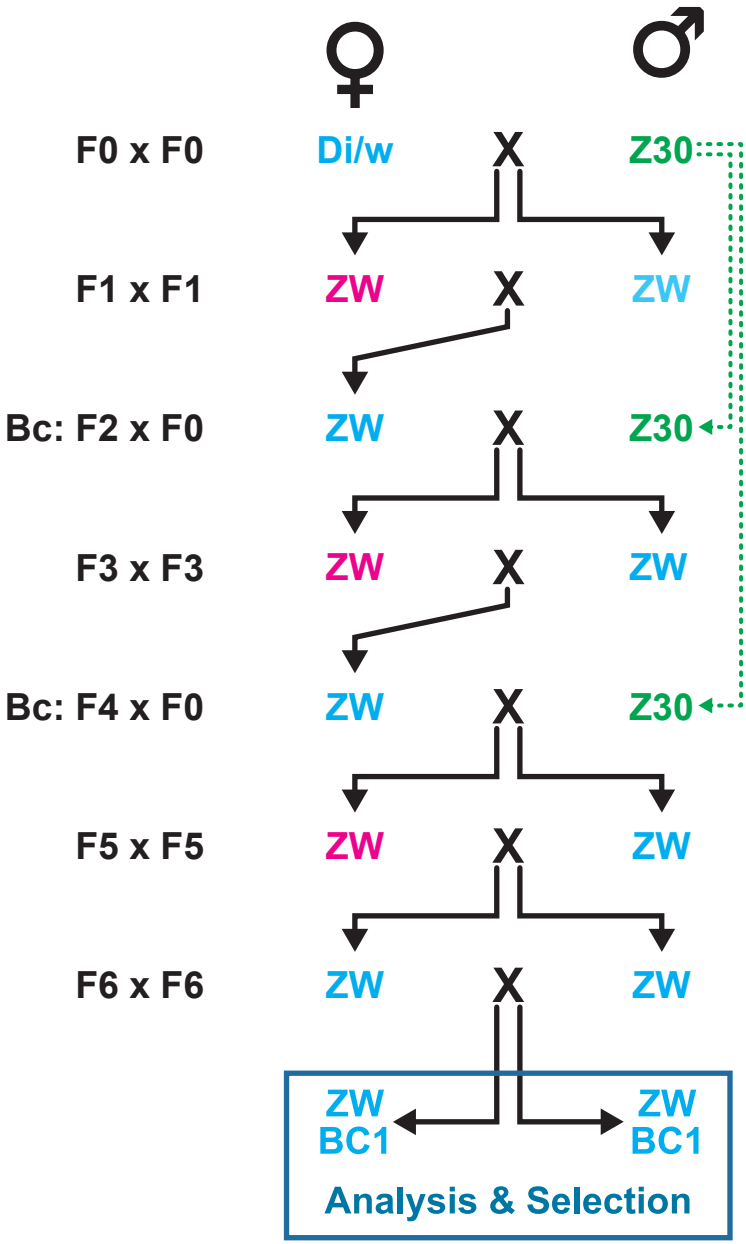
Bars indicate the level and the sign of the correlation between (**A**) the 7/5 ratio (7,11HD/5,9HD in ZW females; 7T/5T in ZW males) and the copulation frequency (dark bars) or the copulation latency (light bars) or (**B**) the copulation frequency and latency. The sex and generation of the pairs of flies tested are indicated under each set of histogram bars. Iso 1W and Iso 3W correspond to four pooled 1W IsoP and four pooled 3W IsoP lines, respectively. The statistical significance is indicated near each corresponding bar.

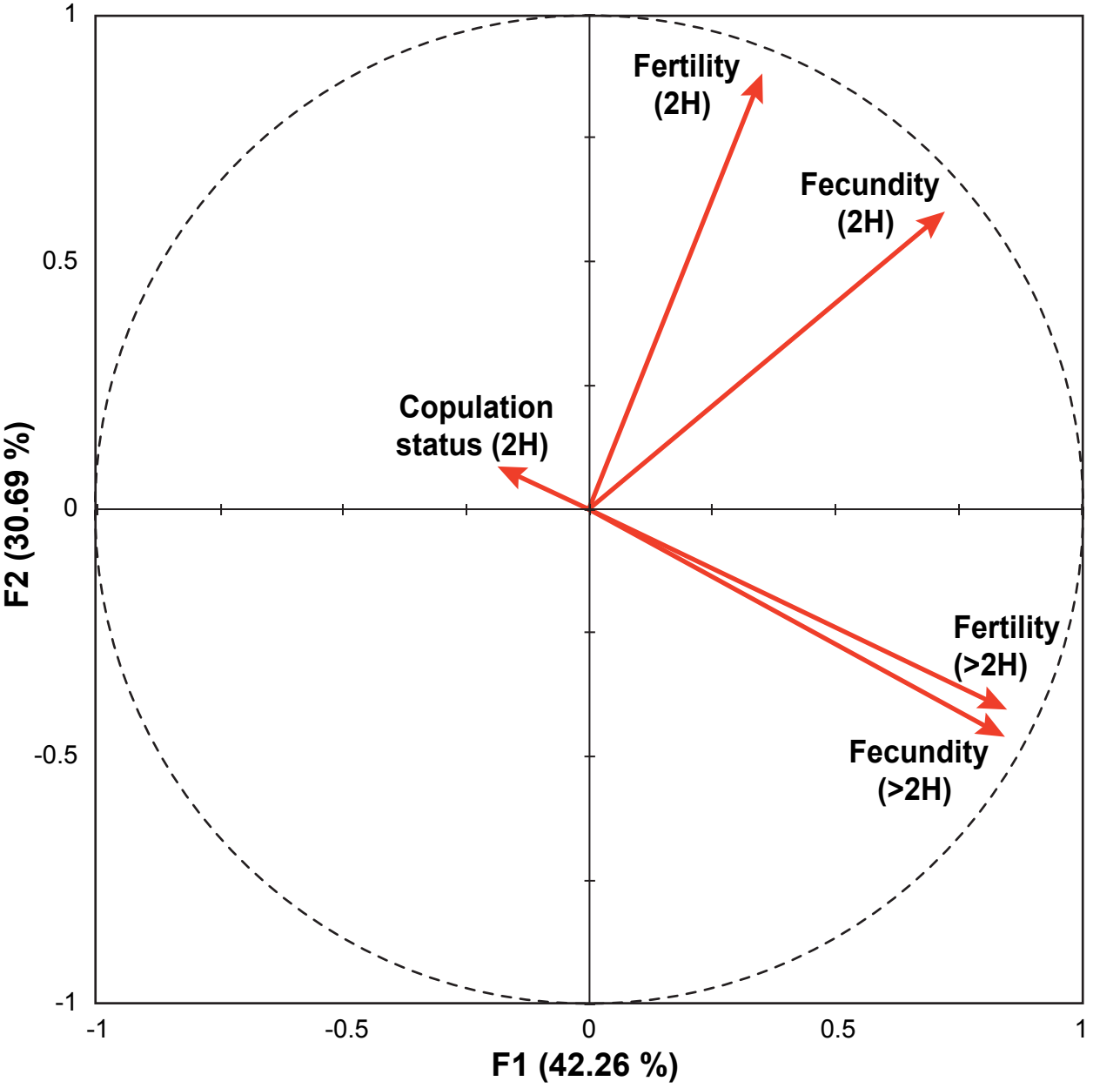
Figure S8. Fecundity in isoparental lines.

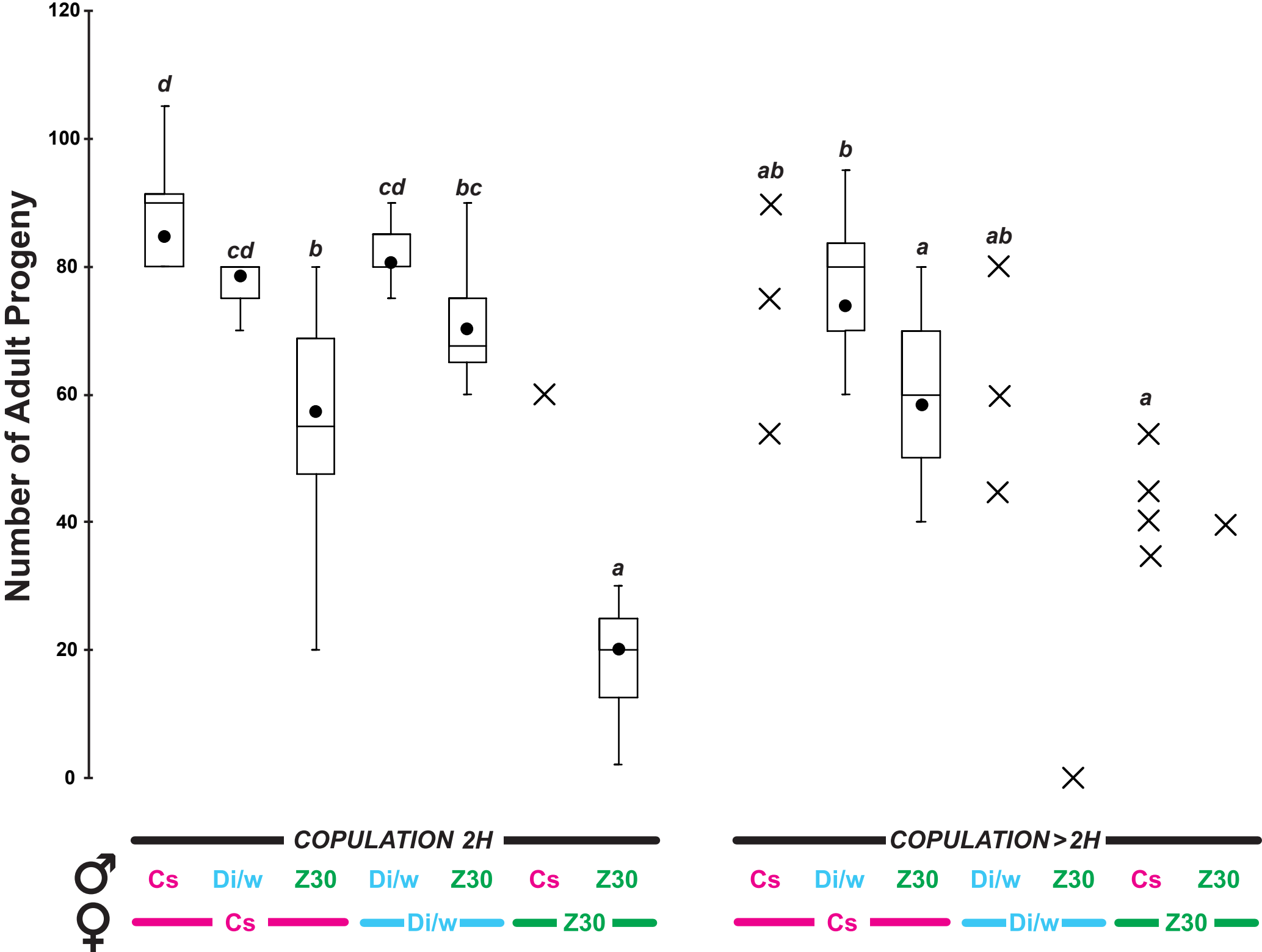
The number of adult progeny left by each pair was counted according to its mating status ("2H": copulation during two-hour period; ">2H": copulation during the following 24-hour period:) and the origin of each parent: females are shown on the top row (under box-plot bars) and males on the bottom row. Flies of isoparental lines (IsoP; four 1W and four 3W IsoP lines) were reciprocally crossed with Z30 flies; ZW-IsoP females were also paired with Cs males and with ZW-IsoP males of the same line. For more information, please refer to Figure S3).

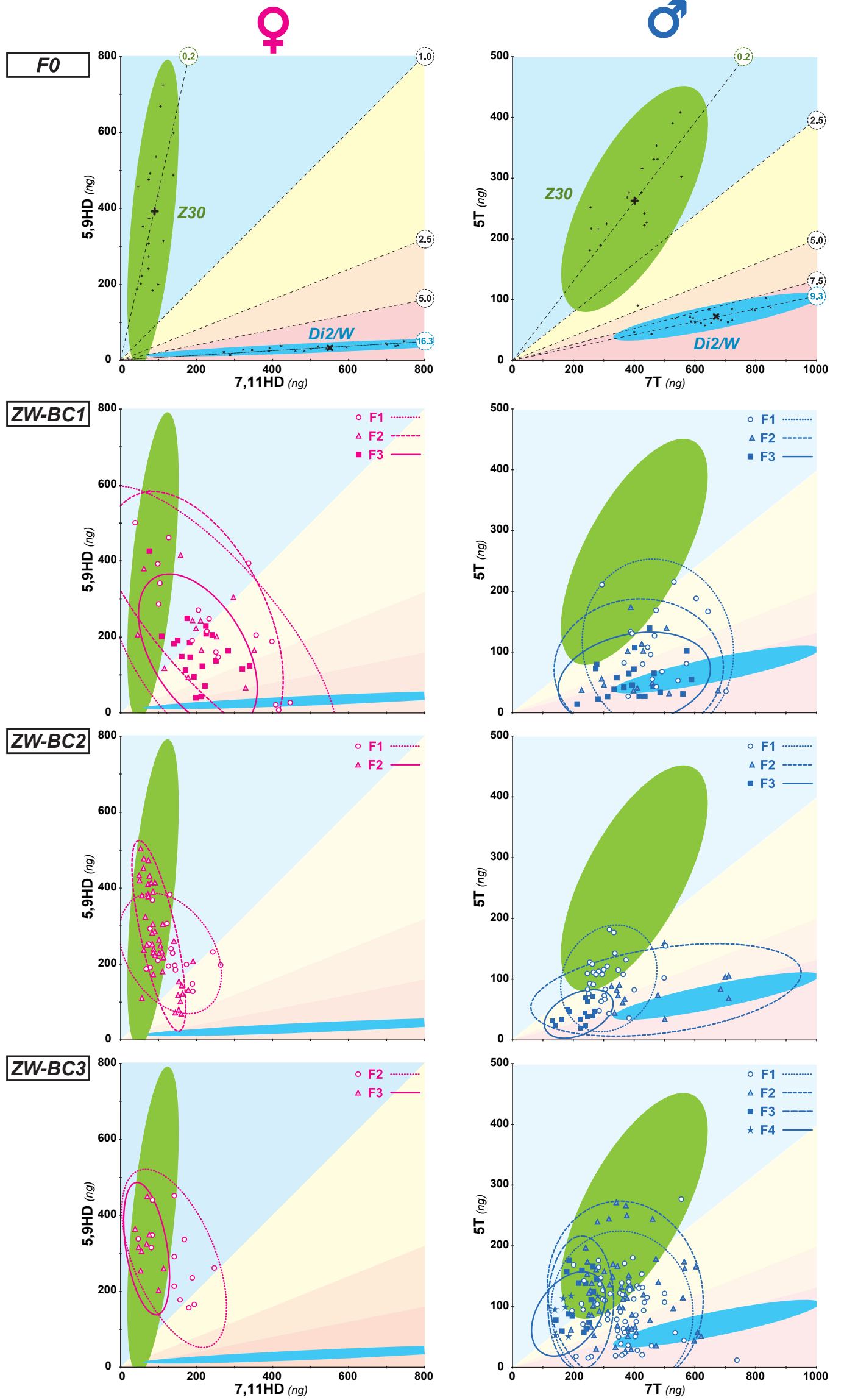
Figure S9. Relationship between cuticular hydrocarbons and copulation performance.

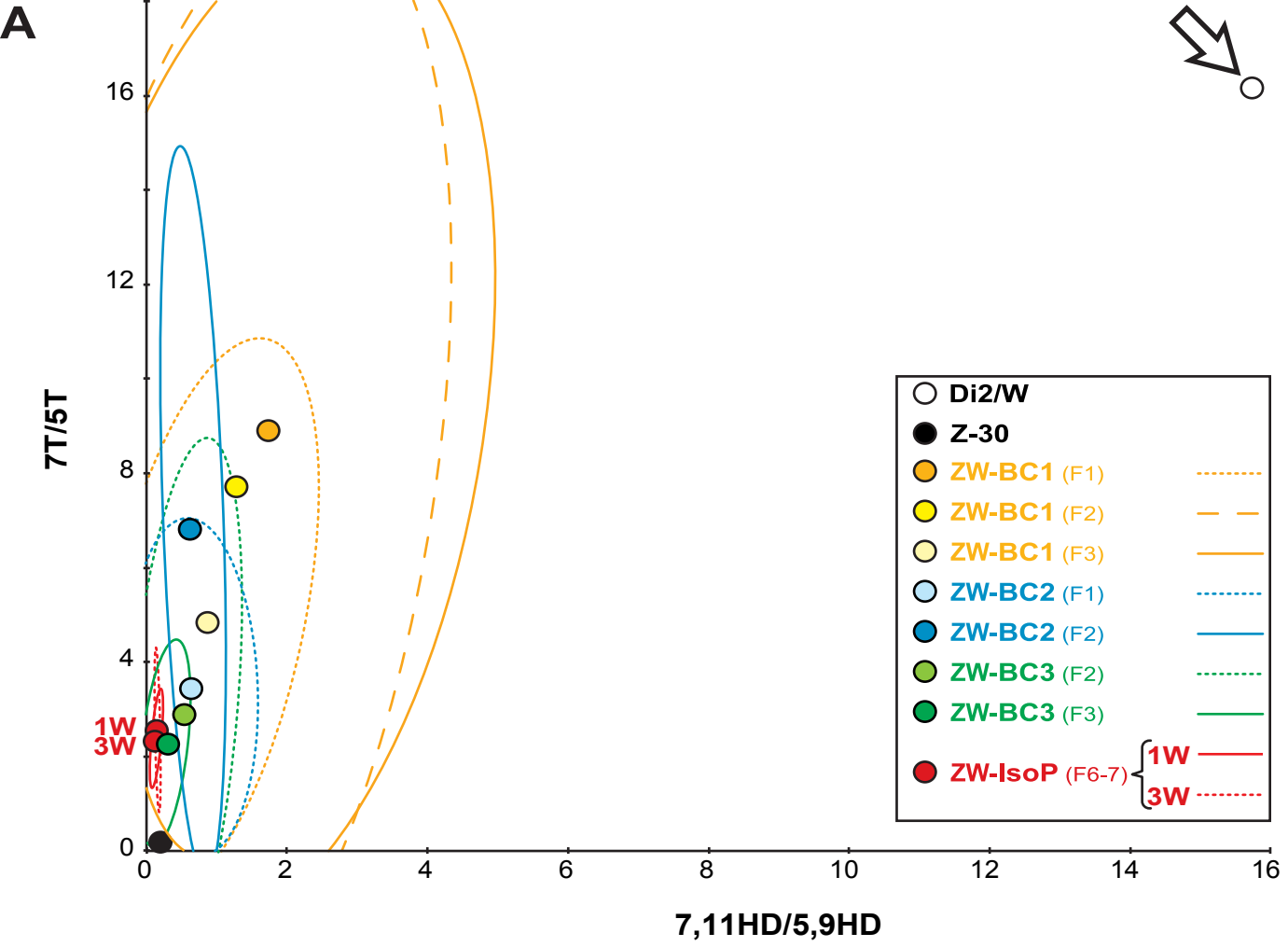
(**A**) The PCA represents the relationship between copulation frequency in females and/or males of eight selected isoparental lines (IsoP) paired with parental flies (Cs, Z30) together with CH ratios (7,11HD/5,9HD in females; 7T/5T in males). A high correlation was found between the 7T/5T ratio and the copulation frequency in ZW-IsoP x Cs (ZW-IsoP females x Cs males) and with ZW-IsoP x ZW-IsoP pairs. Another high correlation was detected between the 7,11HD/5,9HD ratio and the copulation frequency of Z30 females paired with ZW-IsoP males (Z30 x ZW-IsoP). The relative variability is indicated for the two principal axes (F1: 32.30%; F2: 22.97%). (**B**) When ZW-BC1, ZW-BC2 and ZW-BC3 (not ZW-IsoP) flies were considered, a very high correlation was found between the frequency of copulation success rate and the 7,11HD/5,9HD ratio. No correlation was found between these two parameters and the copulation latency or with the 7T/5T ratio; the two latter parameters were not correlated together. The amount of variability for the two principal axis is indicated (F1: 44.2%; F2:30.7%). The distributions of male and female cuticular hydrocarbons ratio are included in ellipses represented with four colors (males) and by plain/dotted lines (in females; see inlet on the right side).

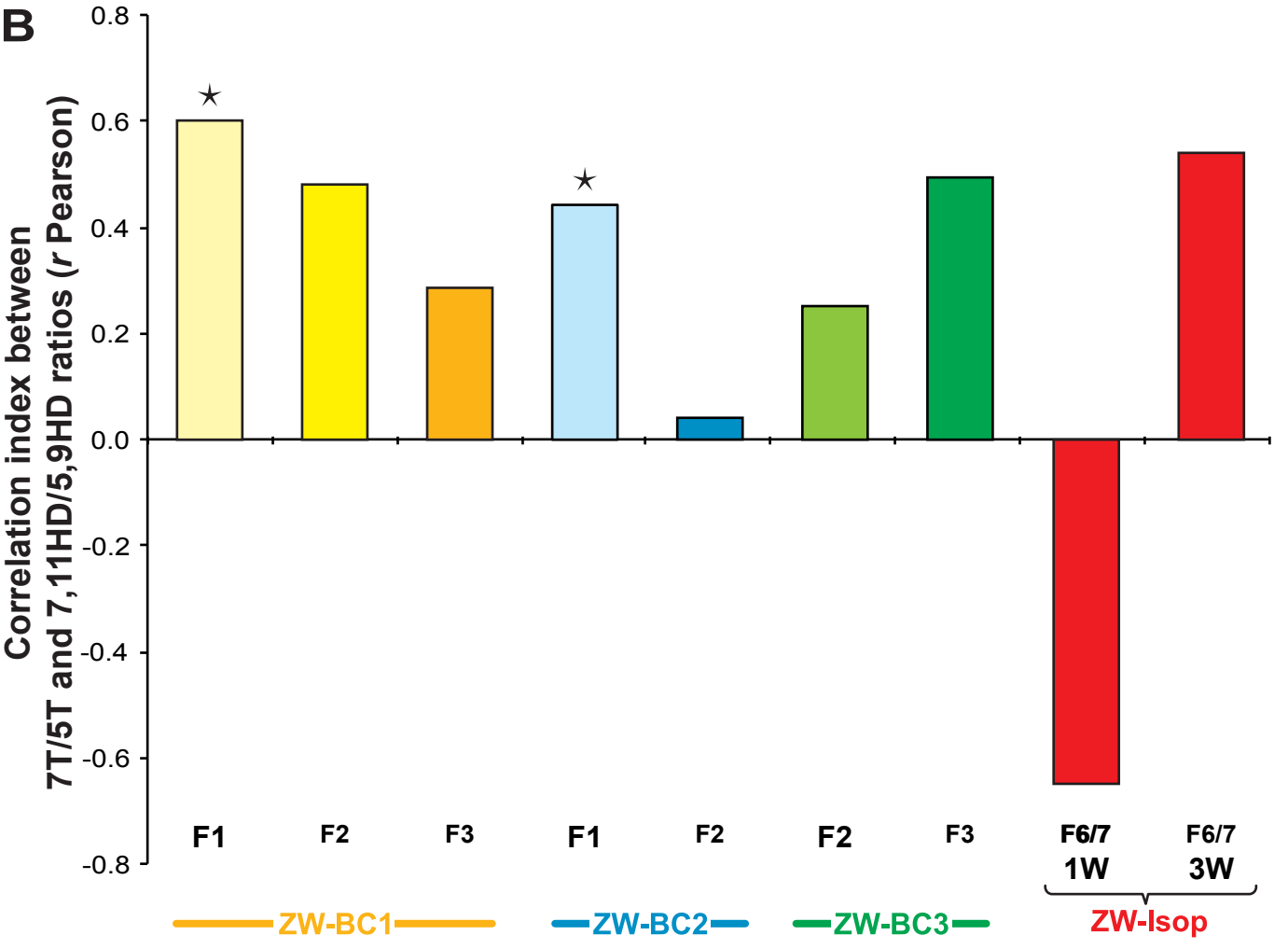

♀
♂
F0 x F0
Di/w
X
Z30
F1 x F1
ZW
X
ZW
Bc: F2 x F0
ZW
X
Z30
F3 x F3
ZW
X
ZW
Bc: F4 x F0
ZW
X
Z30
F5 x F5
ZW
X
ZW
F6 x F6
ZW
X
ZW
ZW
BC1
ZW
BC1
Analysis & Selection

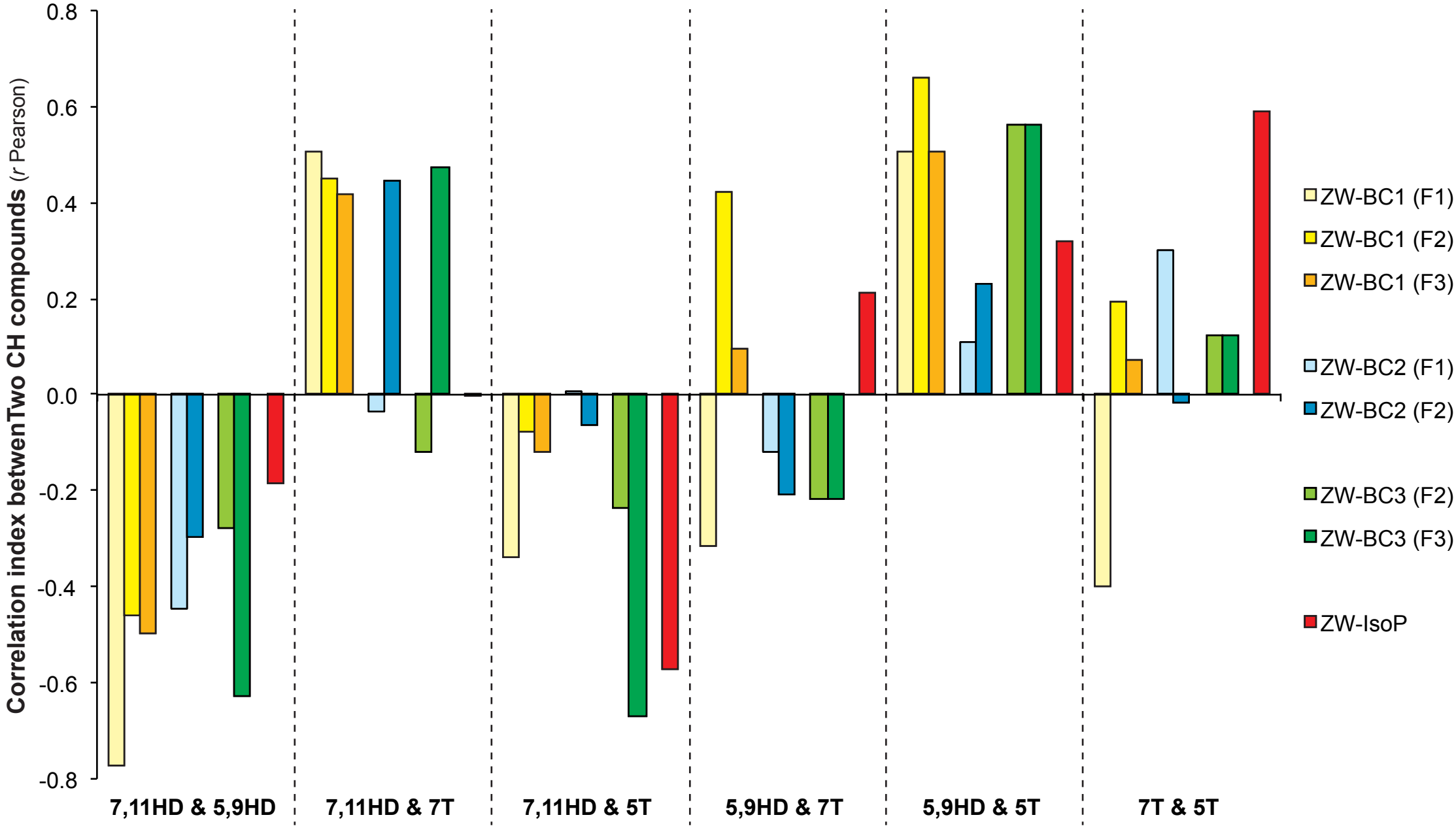

Fertility
(2H)
Fecundity
(2H)
Copulation
status (2H)
Fertility
(>2H)
Fecundity
(>2H)
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
F2 (30.69 %)
F1 (42.26 %)

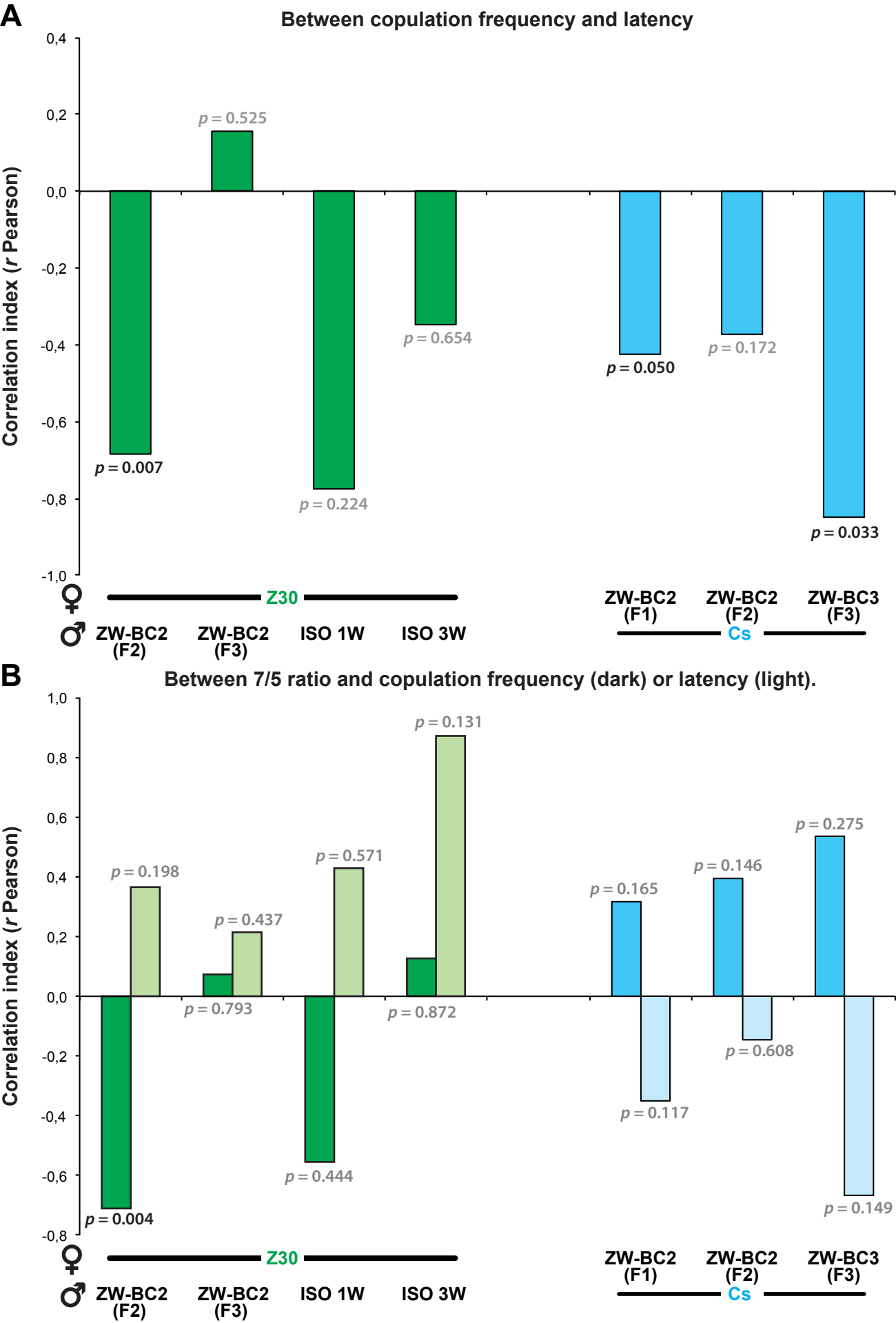

Number of Adult Progeny
120
100
80
60
40
20
0
d
cd
b
cd
bc
a
ab
b
a
ab
a
COPULATION 2H
COPULATION>2H
♂
♀
Cs
Di/w
Z30
Di/w
Z30
Cs
Z30
Cs
Di/w
Z30
Di/w
Z30
Cs
Z30
Cs
Cs
Di/w
Z30

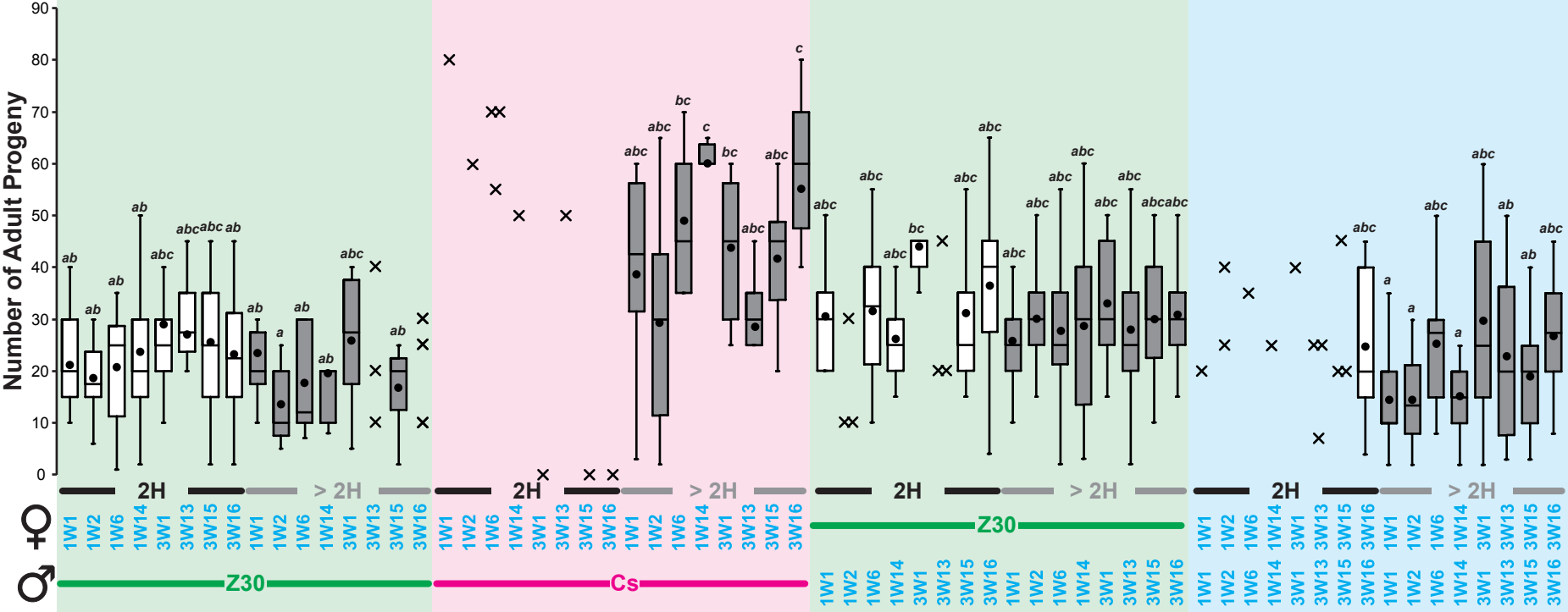

♀
♂
F0
ZW-BC1
ZW-BC2
ZW-BC3
Z30
Di2/W
0.2
1.0
2.5
5.0
16.3
7.5
9.3
F1
F2
F3
F4
5,9HD (ng)
7,11HD (ng)
5T (ng)
7T (ng)
0
200
400
600
800
1000
100
300
500

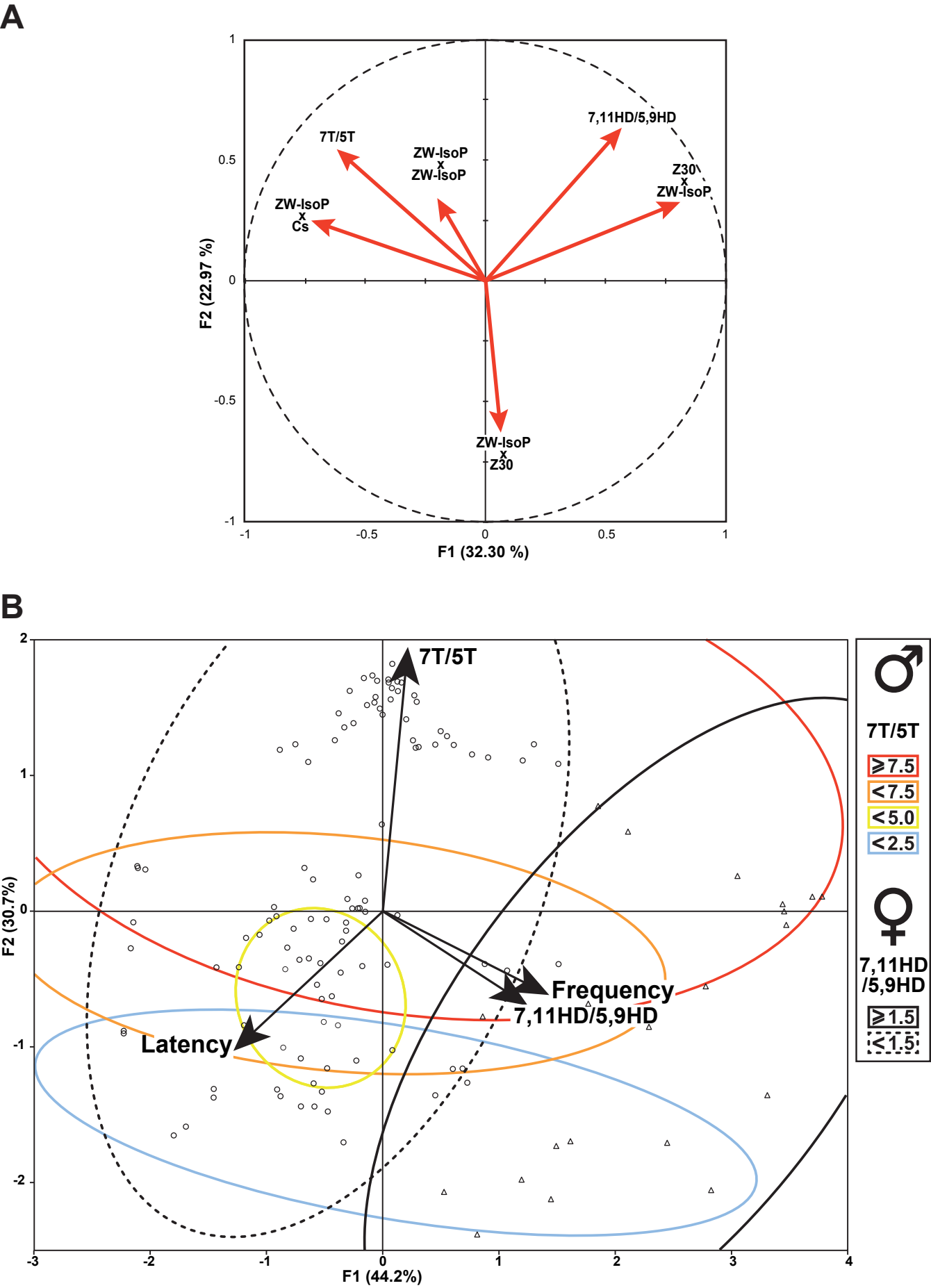

A
7T/5T
7,11HD/5,9HD
0
2
4
6
8
10
12
14
16
1W
3W
Di2/W
Z-30
ZW-BC1 (F1)
ZW-BC1 (F2)
ZW-BC1 (F3)
ZW-BC2 (F1)
ZW-BC2 (F2)
ZW-BC3 (F2)
ZW-BC3 (F3)
ZW-IsoP (F6-7)
1W
3W


B
Correlation index between
7T/5T and 7,11HD/5,9HD ratios (r Pearson)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
F1
F2
F3
F1
F2
F2
F3
F6/7
1W
F6/7
3W
ZW-BC1
ZW-BC2
ZW-BC3
ZW-Isop


0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Correlation index betwenTwo CH compounds (r Pearson)
7,11HD & 5,9HD
7,11HD & 7T
7,11HD & 5T
5,9HD & 7T
5,9HD & 5T
7T & 5T
ZW-BC1 (F1)
ZW-BC1 (F2)
ZW-BC1 (F3)
ZW-BC2 (F1)
ZW-BC2 (F2)
ZW-BC3 (F2)
ZW-BC3 (F3)
ZW-IsoP


A
Between copulation frequency and latency
Correlation index (r Pearson)
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
p = 0.007
p = 0.525
p = 0.224
p = 0.654
p = 0.050
p = 0.172
p = 0.033
Z30
ZW-BC2 (F2)
ZW-BC2 (F3)
ISO 1W
ISO 3W
ZW-BC2 (F1)
ZW-BC2 (F2)
ZW-BC3 (F3)
Cs
B
Between 7/5 ratio and copulation frequency (dark) or latency (light).
Correlation index (r Pearson)
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
p = 0.004
p = 0.198
p = 0.437
p = 0.793
p = 0.571
p = 0.444
p = 0.131
p = 0.872
p = 0.165
p = 0.117
p = 0.146
p = 0.608
p = 0.275
p = 0.149
Z30
ZW-BC2 (F2)
ZW-BC2 (F3)
ISO 1W
ISO 3W
ZW-BC2 (F1)
ZW-BC2 (F2)
ZW-BC3 (F3)
Cs


Number of Adult Progeny
90
80
70
60
50
40
30
20
10
0
2H
> 2H
1W1 1W2 1W6 1W14 3W1 3W13 3W15 3W16
Z30
Cs


A
7T/5T
ZW-IsoP x ZW-IsoP
7,11HD/5,9HD
Z30 x ZW-IsoP
ZW-IsoP x Cs
ZW-IsoP x Z30
F2 (22.97 %)
F1 (32.30 %)
B
7T/5T
Frequency
7,11HD/5,9HD
Latency
F2 (30.7%)
F1 (44.2%)
7T/5T
≥7.5
<7.5
<5.0
<2.5
7,11HD /5,9HD
≥1.5
<1.5