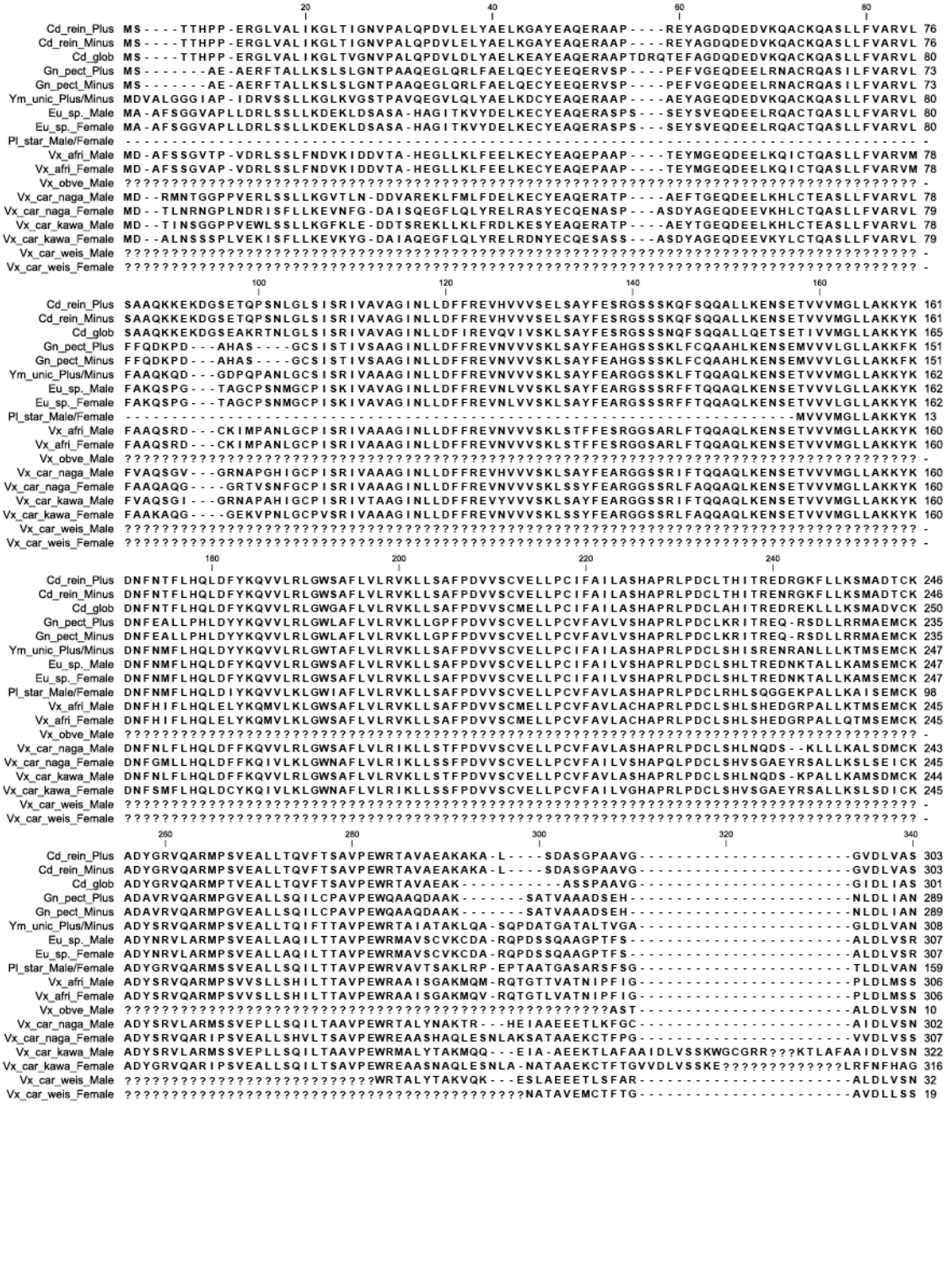
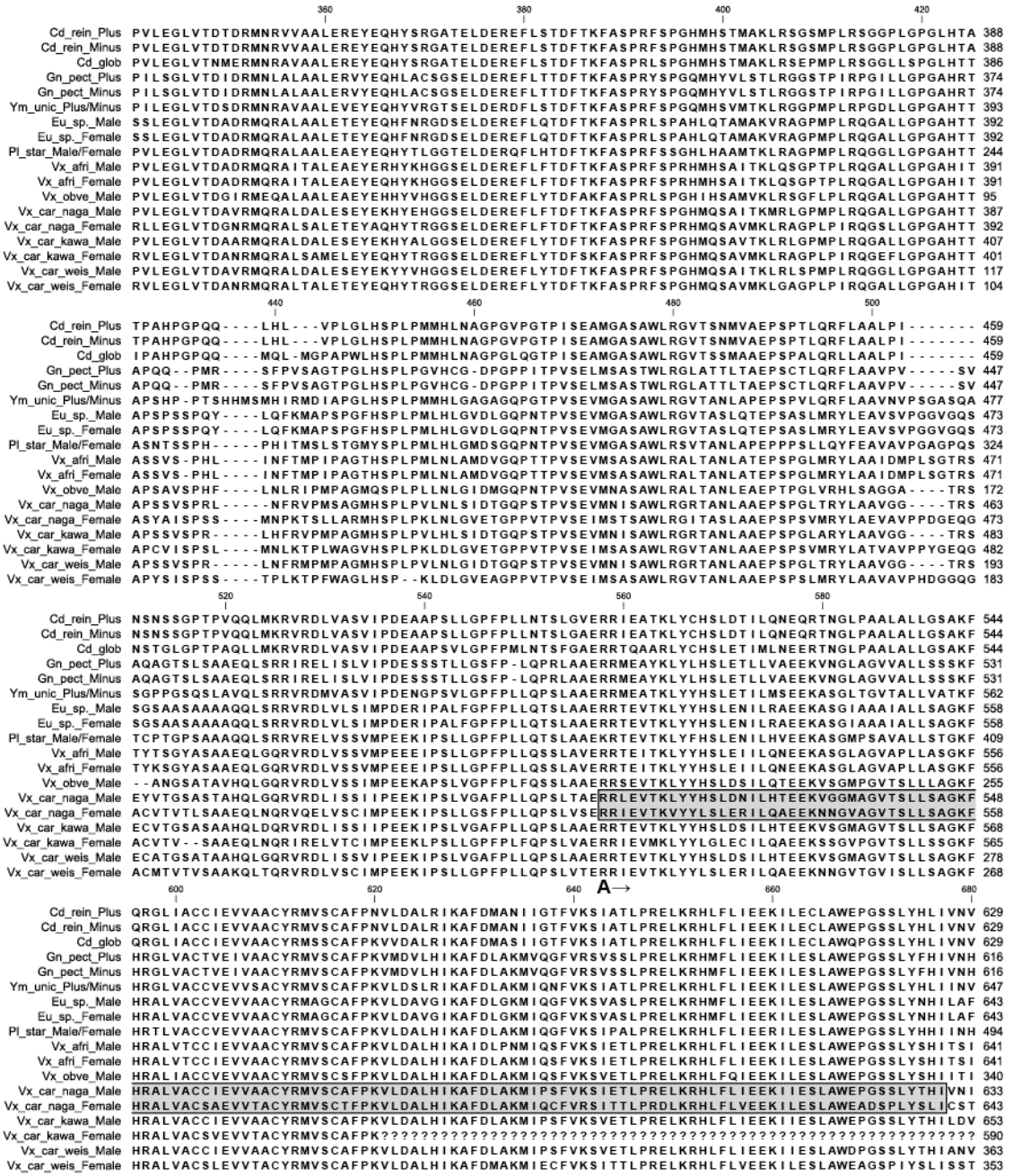
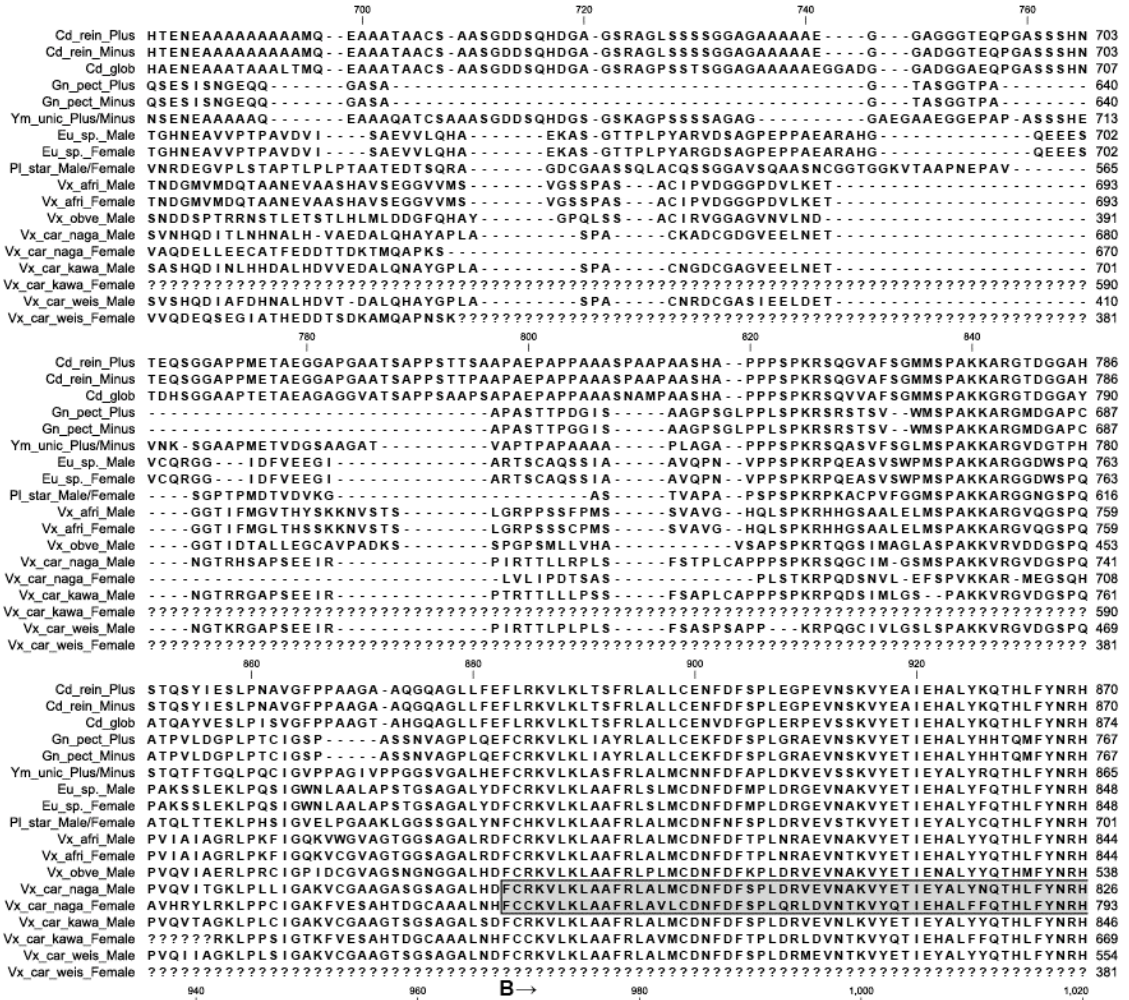
Supplementary Figures




A→


B→

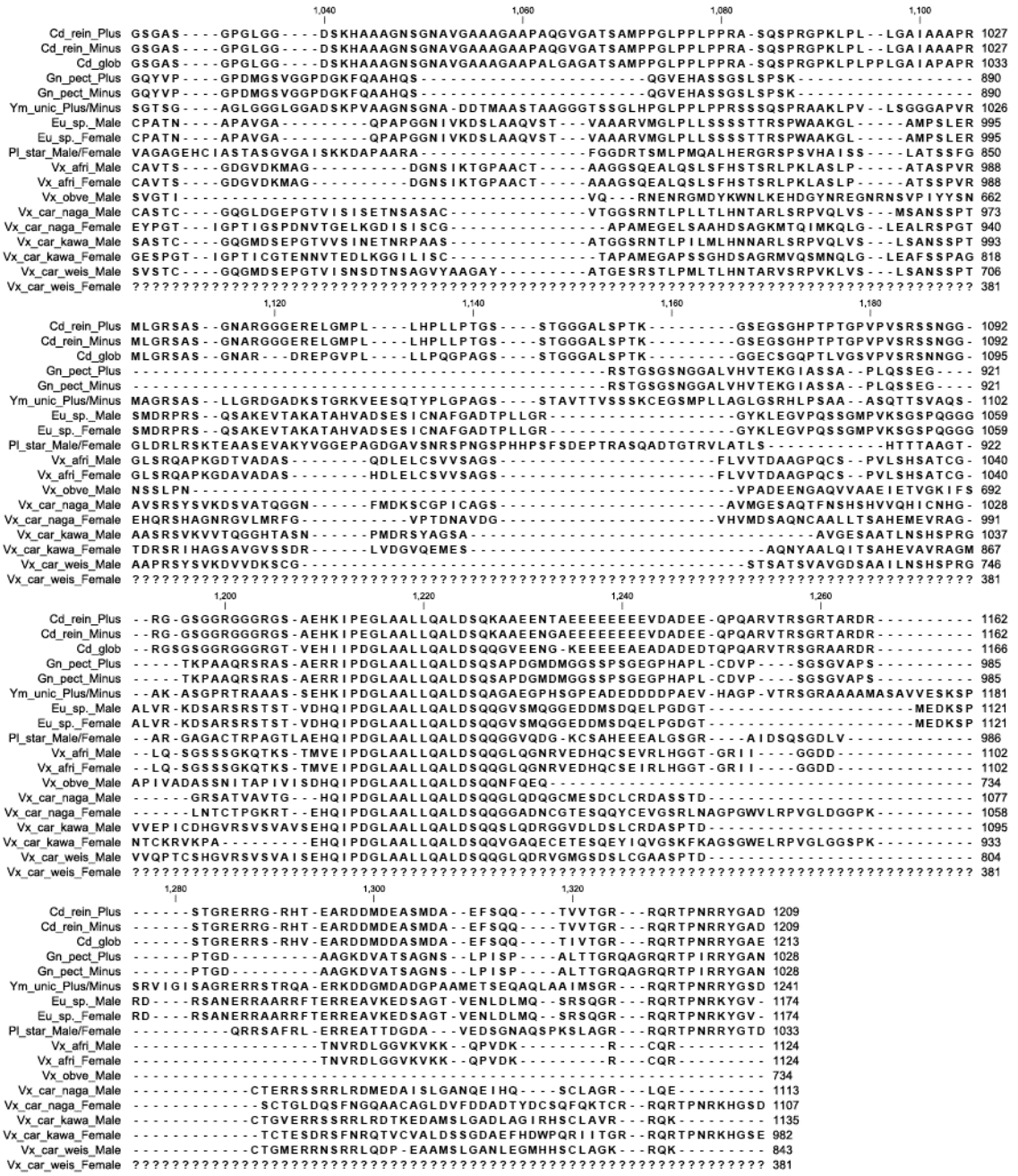
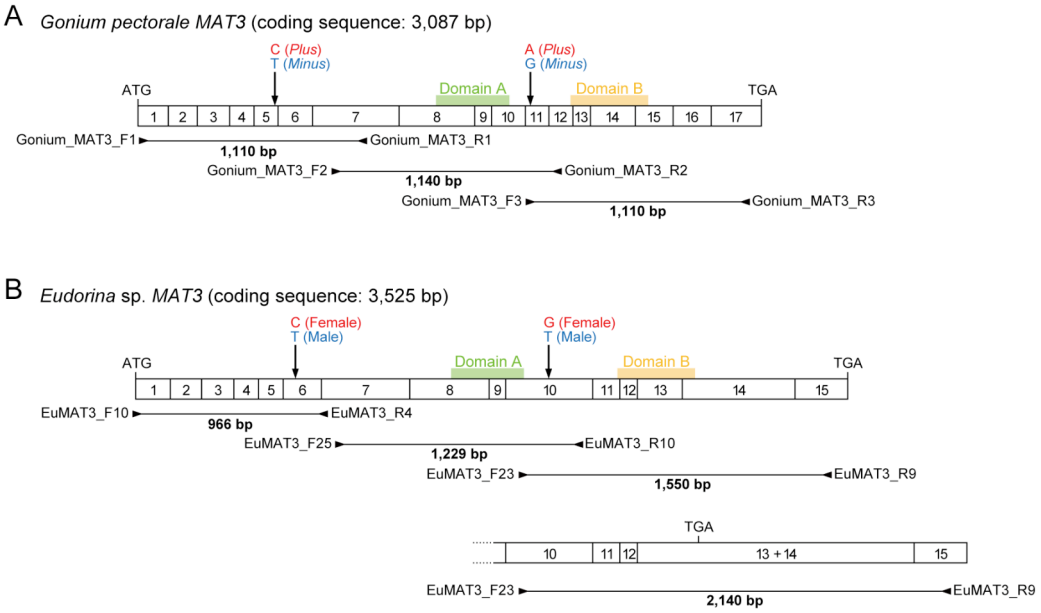
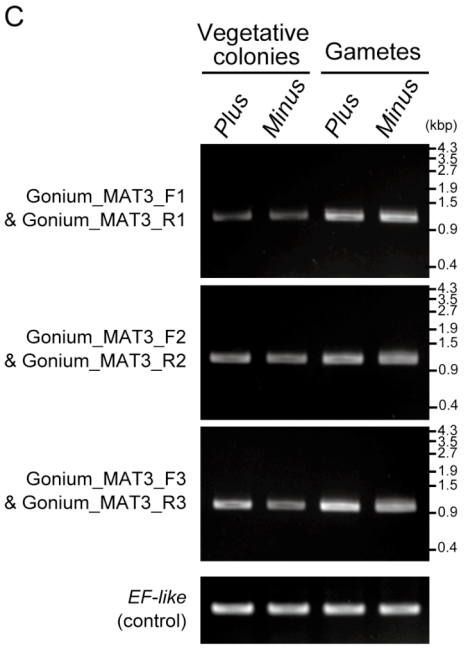


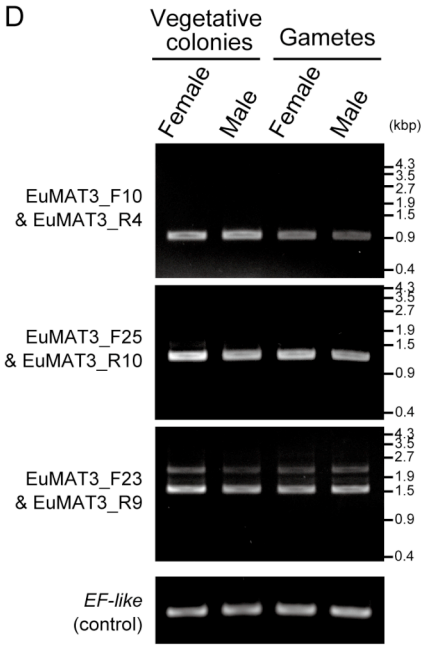
Figure S1

Alignment of full-length MAT3 proteins from isogametic mating type *plus* and *minus* or male and female from six species of the colonial Volvocales, *Chlamydomonas reinhardtii* and *C. globosa* (previously misidentified as *C. incerta* [S1]), plus partial MAT3 proteins from *V. obversus* (male), *V. carteri* f. *weismannia* (male and female) and *V. carteri* f. *kawasakiensis* (male and female) (supplementary table S1, Supplementary Material online). MAT3/RB-specific regions (boxes, Domains A and B)

are shown for *V. carteri* f. *nagariensis*. Positions 341-427, 447-503, 518-678, 874-969, 972-1016 and 1210-1227 (464 amino acids) were used for phylogenetic analysis (fig. 3).


A
Gonium pectorale MAT3 (coding sequence: 3,087 bp)
C (Plus)
T (Minus)
A (Plus)
G (Minus)
ATG
Domain A
Domain B
TGA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Gonium_MAT3_F1
Gonium_MAT3_R1
1,110 bp
Gonium_MAT3_F2
Gonium_MAT3_R2
1,140 bp
Gonium_MAT3_F3
Gonium_MAT3_R3
1,110 bp
B
Eudorina sp. MAT3 (coding sequence: 3,525 bp)
C (Female)
T (Male)
G (Female)
T (Male)
ATG
Domain A
Domain B
TGA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
EuMAT3_F10
EuMAT3_R4
966 bp
EuMAT3_F25
EuMAT3_R10
1,229 bp
EuMAT3_F23
EuMAT3_R9
1,550 bp
TGA
10 11 12 13 + 14 15
EuMAT3_F23
EuMAT3_R9
2,140 bp


C
Vegetative colonies
Gametes
Plus
Minus
Plus
Minus
(kbp)
Gonium_MAT3_F1 & Gonium_MAT3_R1
Gonium_MAT3_F2 & Gonium_MAT3_R2
Gonium_MAT3_F3 & Gonium_MAT3_R3
EF-like (control)
4.3
3.5
2.7
1.9
1.5
0.9
0.4


D
Vegetative colonies
Gametes
Female
Male
Female
Male
(kbp)
EuMAT3_F10 & EuMAT3_R4
EuMAT3_F25 & EuMAT3_R10
EuMAT3_F23 & EuMAT3_R9
EF-like (control)
4.3
3.5
2.7
1.9
1.5
0.9
0.4

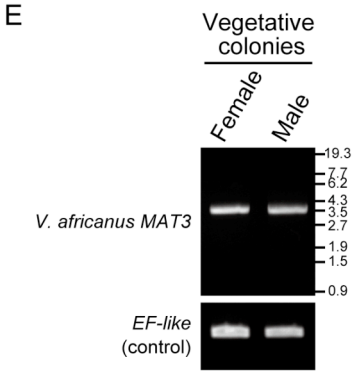

E
Vegetative colonies
Female
Male
V. africanus MAT3
EF-like (control)
19.3
7.7
6.2
4.3
3.5
2.7
1.9
1.5
0.9

Figure S2

Semi-quantitative RT-PCR analyses of *MAT3* from different colonial Volvocales. (*A, B*) Schematic representations of *Gonium pectorale* (*A*) and *Eudorina* sp. (*B*) *MAT3* coding sequences. Numbers in the open boxes indicate the exon numbers. The regions corresponding to the two conserved domains A and B are shown. The positions of the bases different between each sex are also indicated. The primer sets (supplementary information, Supplementary Material online) used in the RT-PCR experiments and their resulting product sizes are given. A partial structure of the *Eudorina* sp. *MAT3* splicing variant identified by sequencing of the PCR product (*D*, upper band amplified with the primer set EuMAT3_F23 and EuMAT3_R9 [supplementary information, Supplementary Material online]) is also shown (*B*). (*C-E*) Results of semi-quantitative RT-PCR analyses of *MAT3* genes from *G. pectorale* (*C*), *Eudorina* sp. (*D*), and *Volvox africanus* (*E)*. Poly(A)$^+$ mRNA from each sample was isolated and subjected to RT-PCR analysis using primer sets indicated. Loading volume for each lane was normalized to the amount of the *EF-like* gene (internal control) product. Note that no significant differences in expression level and splicing pattern were detected between the two sexes. For *V. africanus* (*E*), a primer set which amplifies a 3,462-bp fragment including the full-length coding sequence of female and male *MAT3* (Vol_Africa_male_F8 and Vol_Africa_CDS_R1 [supplementary information, Supplementary Material online]) was used for the analysis.

Supplementary Reference

S1. Nakada T, Shinkawa H, Ito T, Tomita M. 2010. Recharacterization of *Chlamydomonas reinhardtii* and its relatives with new isolates from Japan. J Plant Res. 123: 67–78.