Electronic Supplementary Material

Supplementary Results

Isogamete versus microgamete volume

For unicellular species, isogamete volume is generally higher than microgamete volume. We tested this pattern by regressing the $\log_{10}$ volumes of isogametes and microgametes on $\log_{10}$ protoplasmic volume and comparing the residuals between isogametes and microgametes (Fig. S3). One isogamous species, *Lobocharacium coloradoense*, has an exceptionally high protoplasmic volume because it is multinucleate, and therefore could be classified as colonial, and for this reason was omitted from the analysis. A one-tail *t*-test, assuming unequal variances, between the residuals of isogamete (mean $\pm$ standard deviation) (0.2709 ± 0.5236 μm^3) and microgamete (-0.4741 ± 0.2476 μm^3) volumes shows that isogametes are significantly larger than microgametes for unicellular species (t = 4.5130, df = 20, P = 0.0001). A similar pattern holds for colonial and multicellular species. Because microgamete volume is not linearly related to protoplasmic volume for these species, $\log_{10}$ volumes were directly compared between isogametes (2.9120 ± 0.4755 μm^3) and microgametes (2.2220 ± 0.7327 μm^3), showing that isogametes are significantly larger (t = 3.0571, df = 26, P = 0.0026). To control for the positive relationship between isogamete and macrogamete volumes and protoplasmic volume for these species, $\log_{10}$ isogamete and macrogamete volumes were regressed on $\log_{10}$ protoplasmic volume, and the residuals of isogamete volume were compared to the differences between microgamete volume and the gamete volume predicted from the regression for the species' protoplasmic volume (Fig. S4). This also shows that isogametes (-0.2912 ± 0.4652 μm^3) are significantly larger than microgametes (-1.7767 ± 0.9215 μm^3) for colonial and multicellular species (t = 5.6260, df = 23, $P = 5.01 \times 10^{-6}$).

Table S1. Gamete chloroplast characteristics of isogamous volvocine algae.

Family	Species	No. of cells	Structural grade	Gamete chloroplast	Ref.
Characiosiphonaceae	*Lobocharacium coloradoense*	1	unicellular	Chloroplast with pyrenoid; Sparse amounts of starch in chloroplast; Chloroplast does not occupy significant area of gamete	[1]
Chlamydomonadaceae	*Carteria palmata*	1	unicellular	Chloroplast containing a pyrenoid and an eyespot	[2]
Chlamydomonadaceae	*Chlamydomonas chlamydogama*	1	unicellular	Chloroplast occupies most of cell; one prominent pyrenoid	[3]
Chlamydomonadaceae	*Chlamydomonas microhalophila*	1	unicellular	Chloroplast occupies large space; pyrenoid present	[4]
Chlamydomonadaceae	*Chlamydomonas moewusii*	1	unicellular	Chloroplast present	[5]
Chlamydomonadaceae	*Chlamydomonas reinhardtii*	1	unicellular	Chloroplast contains an abundance of starch; pyrenoid present	[6, 7]
Chlamydomonadaceae	*Lobochlamys segnis*	1	unicellular	Dissected plastid with faintly visible pyrenoids	[8]
Chlamydomonadaceae	*Pseudocarteria corcontica*	1	unicellular	Chloroplast with one pyrenoid	[9]
Chlorosarcinaceae	*Desmotetra antarctica*	16	colonial	Chloroplast present	[10]
Chlorosarcinaceae	*Desmotetra aureospora*	16	colonial	Small discoid chloroplast	[10]
Goniaceae	*Gonium sociale*	4	colonial	Chloroplast with one pyrenoid	[11]
Goniaceae	*Gonium quadratum*	16	colonial	Chloroplast with one pyrenoid	[12]
Goniaceae	*Gonium viridistellatum*	16	colonial	Massive chloroplast with one pyrenoid	[13]
Goniaceae	*Gonium multicoccum*	32	colonial	Massive chloroplast	[14]
Tetrabaenaceae	*Basichlamys sacculifera*	4	colonial	Chloroplast with one pyrenoid	[11]
Volvocaceae	*Volvulina compacta*	16	colonial	Massive chloroplast, up to 20µm dia. (cell ~21µm dia.) with a single large basal pyrenoid with many associated starch grains	[15, 16]
Volvocaceae	*Volvulina pringsheimii*	16	colonial	Massive chloroplast	[17]
Volvocaceae	*Pandorina unicocca*	32	colonial	Massive chloroplast; Chloroplast fills most of posterior part of cell and periphery of anterior part of cell; pyrenoid is surrounded by several starch grains	[18, 19]
Volvocaceae	*Volvulina steinii*	32	colonial	Chloroplast without pyrenoid	[20]
Volvocaceae	*Astrephomene gubernaculifera*	128	multicellular	Chloroplast present	[20]

Table S2. Gamete chloroplast characteristics of anisogamous volvocine algae.

Family	Species	No. of cells	Structural grade	Gamete dimorphism*	Microgamete chloroplast	Macrogamete chloroplast	Ref.
Chlamydomonadaceae	*Chlamydomonas allensworthii*	1	unicellular	anisogamy	No information	Photosynthetic system deconstructed to produce chemoattractant; chloroplast fades from green to brownish	[21]
Chlamydomonadaceae	*Chlamydomonas sphagnicola*	1	unicellular	oogamy	Smaller than the macrogamete chloroplast; one pyrenoid	Large compared to microgamete chloroplast; four pyrenoids	[22]
Chlamydomonadaceae	*Chlamydomonas suboogama*	1	unicellular	anisogamy	Smaller than macrogamete chloroplast; no pyrenoid	Larger than microgamete chloroplast; covers large area of gamete; one large pyrenoid	[22]
Chlamydomonadaceae	*Oogamochlamys ettlii*	1	unicellular	oogamy	Chloroplast reduced and pale green; no pyrenoid	No information	[23]
Chlamydomonadaceae	*Oogamochlamys gigantea*	1	unicellular	oogamy	Chloroplast reduced and pale green or yellow-tinged; no pyrenoid;	Large in comparison to microgamete chloroplast; occupies most of cell; five pyrenoids	[22, 23]
Chlamydomonadaceae	*Oogamochlamys zimbabwiensis*	1	unicellular	oogamy	Chloroplast reduced; pale green; no pyrenoid	No information	[23]
Volvocaceae	*Eudorina echidna*	16	colonial	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	Chloroplast with a pyrenoid	[24]
Volvocaceae	*Colemanosphaera charkowiensis*	32	colonial	anisogamy	No information	Chloroplast with 3-8 pyrenoids	[25]
Volvocaceae	*Eudorina cylindrica*	32	colonial	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	Chloroplast with one large central or basal pyrenoid and one to several smaller peripheral pyrenoids	[24]
Volvocaceae	*Eudorina elegans*	32	colonial	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	Multiple pyrenoids in chloroplast	[24, 26]
Volvocaceae	*Eudorina illinoisensis*	32	colonial	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	Chloroplast with one to several pyrenoids	[24]
Volvocaceae	*Eudorina unicocca*	32	colonial	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	Chloroplast with one large central or basal pyrenoid	[24]

Family	Species	No. of cells	Structural grade	Gamete dimorphism*	Microgamete chloroplast	Macrogamete chloroplast	Ref.
Volvocaceae	*Pleodorina starrii*	64	multicellular	anisogamy (I)	No information	Massive chloroplast with basal pyrenoid surrounded by starch grains; fills most of cell periphery	[27]
Volvocaceae	*Pleodorina californica*	128	multicellular	anisogamy (I)	Yellow-green chloroplast occupies lower half of cell	One to many pyrenoids present	[24]
Volvocaceae	*Pleodorina japonica*	128	multicellular	anisogamy (I)	No information	Chloroplast with pyrenoids	[28]
Volvocaceae	*Pleodorina sphaerica*	128	multicellular	anisogamy (I)	No information	Massive chloroplast with 4-10 pyrenoids	[29]
Volvocaceae	*Volvox tertius*	2,000	multicellular	oogamy (I)	No chlorophyll in microgamete	No information	[30]
Volvocaceae	*Volvox carteri*	3,000	multicellular	oogamy (I)	Large chloroplast contains zero to several condensed nuclei	Enlarged chloroplast with pyrenoids	[31]
Volvocaceae	*Volvox aureus*	3,200	multicellular	oogamy (I)	No information	Chloroplast with pyrenoids	[30]

*(I) indicates internal fertilization.

Table S3. Protoplasm and gamete volumes for volvocine algae. Data are primarily from da Silva [32], with additional species from other sources [22, 29, 33-35].

Family	Species	No. of cells	Structural grade	Gamete dimorphism*	Protoplasm volume (µm^3)	Macro- or iso-gamete volume (µm^3), *b*	Micro- or iso-gamete volume (µm^3), *a*	*b*/*a*
Characiosiphonaceae	*Lobocharacium coloradoense*	1	unicellular	isogamy	1734159	524	524	1
Chlamydomonadaceae	*Carteria eugametos*	1	unicellular	isogamy	785	785	785	1
Chlamydomonadaceae	*Carteria palmata*	1	unicellular	isogamy	5080	5080	5080	1
Chlamydomonadaceae	*Chlamydomonas acidophila*	1	unicellular	isogamy	1670	104	104	1
Chlamydomonadaceae	*Chlamydomonas allensworthii*	1	unicellular	anisogamy	950	950	59	16
Chlamydomonadaceae	*Chlamydomonas chlamydogama*	1	unicellular	isogamy	1120	560	560	1
Chlamydomonadaceae	*Chlamydomonas indica*	1	unicellular	isogamy	733	183	183	1
Chlamydomonadaceae	*Chlamydomonas iyengarii*	1	unicellular	isogamy	760	760	760	1
Chlamydomonadaceae	*Chlamydomonas microhalophila*	1	unicellular	isogamy	1508	754	754	1
Chlamydomonadaceae	*Chlamydomonas moewusii*	1	unicellular	isogamy	8370	2093	2093	1
Chlamydomonadaceae	*Chlamydomonas pitschmannii*	1	unicellular	isogamy	873	55	55	1
Chlamydomonadaceae	*Chlamydomonas reinhardtii*	1	unicellular	isogamy	11000	5500	5500	1
Chlamydomonadaceae	*Chlamydomonas sphagnicola*	1	unicellular	oogamy	3591	3591	225	16
Chlamydomonadaceae	*Chlamydomonas suboogama*	1	unicellular	anisogamy	3054	3054	191	16
Chlamydomonadaceae	*Chloromonas chenangoensis*	1	unicellular	isogamy	4009	4009	4009	1
Chlamydomonadaceae	*Lobochlamys segnis*	1	unicellular	isogamy	700	22	22	1
Chlamydomonadaceae	*Microglena coccifera*	1	unicellular	oogamy	10306	10306	644	16
Chlamydomonadaceae	*Microglena monadina*	1	unicellular	anisogamy	6004	3002	751	4
Chlamydomonadaceae	*Oogamochlamys ettlii*	1	unicellular	oogamy	6842	6842	428	16
Chlamydomonadaceae	*Oogamochlamys gigantea*	1	unicellular	oogamy	8181	8181	128	64
Chlamydomonadaceae	*Oogamochlamys zimbabwiensis*	1	unicellular	oogamy	4189	4189	262	16
Chlamydomonadaceae	*Pseudocarteria corcontica*	1	unicellular	isogamy	13360	13360	13360	1
Chlorosarcinaceae	*Desmotetra antarctica*	16	colonial	isogamy	147244	575	575	1

Family	Species	No. of cells	Structural grade	Gamete dimorphism*	Protoplasm volume (μm³)	Macro- or iso-gamete volume (μm³), *b*	Micro- or iso-gamete volume (μm³), *a*	*b*/*a*
Chlorosarcinaceae	*Desmotetra aureospora*	16	colonial	isogamy	77584	303	303	1
Goniaceae	*Gonium maiaprilis*	16	colonial	isogamy	28272	221	221	1
Goniaceae	*Gonium pectorale*	16	colonial	isogamy	75600	2363	2363	1
Goniaceae	*Gonium quadratum*	16	colonial	isogamy	11200	697	697	1
Goniaceae	*Gonium viridistellatum*	16	colonial	isogamy	28300	884	884	1
Goniaceae	*Gonium multicoccum*	32	colonial	isogamy	97700	3053	3053	1
Goniaceae	*Astrephomene gubernaculifera*	128	multicellular	isogamy	6100000	3050	3050	1
Haematococcaceae	*Chlorogonium mariae*	1	unicellular	isogamy	524	131	131	1
Haematococcaceae	*Stephanosphaera pluvialis*	8	colonial	isogamy	10648	83	83	1
Tetrabaenaceae	*Tetrabaena socialis*	4	colonial	isogamy	11800	738	738	1
Volvocaceae	*Volvulina pringsheimii*	16	colonial	isogamy	28300	1767	1767	1
Volvocaceae	*Colemanosphaera charkowiensis*	32	colonial	anisogamy	82318	2572	80	32
Volvocaceae	*Eudorina elegans*	32	colonial	anisogamy (I)	232000	3625	57	64
Volvocaceae	*Pandorina morum*	32	colonial	isogamy	294000	574	574	1
Volvocaceae	*Platydorina caudata*	32	colonial	anisogamy	262000	4094	128	32
Volvocaceae	*Volvulina steinii*	32	colonial	isogamy	70600	2206	2206	1
Volvocaceae	*Pleodorina indica*	64	multicellular	anisogamy (I)	357000	1150	18	64
Volvocaceae	*Pleodorina japonica*	128	multicellular	anisogamy (I)	1040000	14100	110	128
Volvocaceae	*Pleodorina sphaerica*	128	multicellular	anisogamy (I)	406943	14137	110	129
Volvocaceae	*Volvox pocockiae*	1500	multicellular	oogamy (I)	2650000	113000	1766	64
Volvocaceae	*Volvox tertius*	2000	multicellular	oogamy (I)	763000	655000	20469	32
Volvocaceae	*Volvox carteri*	3000	multicellular	oogamy (I)	1570000	10300	80	128
Volvocaceae	*Volvox aureus*	3200	multicellular	oogamy (I)	362000	7230	226	32
Volvocaceae	*Volvox obversus*	4000	multicellular	oogamy (I)	2090000	38800	303	128
Volvocaceae	*Volvox africanus*	6000	multicellular	oogamy (I)	963000	47700	373	128

Family	Species	No. of cells	Structural grade	Gamete dimorphism*	Protoplasm volume (µm³)	Macro- or iso-gamete volume (µm³), *b*	Micro- or iso-gamete volume (µm³), *a*	*b*/*a*
Volvocaceae	*Volvox capensis*	20000	multicellular	oogamy (I)	42100000	125000	244	512
Volvocaceae	*Volvox globator*	22000	multicellular	oogamy (I)	737000	11000	43	256
Volvocaceae	*Volvox dissipatrix*	31800	multicellular	oogamy (I)	133000000	33500	131	256
Volvocaceae	*Volvox rousseletii*	50000	multicellular	oogamy (I)	105000000	14100	28	512

*(I) indicates internal fertilisation.

Table S4. Phylogenetically independent contrasts of the slope of log anisogamy ratio (*b*/*a*) on log protoplasmic volume for anisogamous taxa.

Contrast	Taxon 1	Log_{10} Protoplasmic volume (μm^3)	Log_{10} *b*/*a*	Taxon 2	Log_{10} Protoplasmic volume (μm^3)	Log_{10} *b*/*a*	Slope
1	*Volvox dissipatrix*	8.1239	2.4082	*Volvox tertius*	5.8825	1.5051	0.4029
2	mean of contrast 1	7.8253	2.1584	*Volvox africanus*	5.9836	2.1072	0.0278
3	*Volvox obversus*	6.3201	2.1072	*Volvox carteri*	6.1959	2.1072	0
4	mean of contrast 2	7.5305	2.1335	mean of contrast 3	6.2625	2.1072	0.4956
5	*Pleodorina japonica*	6.0170	2.1072	*Volvox aureus*	5.5587	1.5051	1.3136
6	mean of contrast 5	5.8457	1.9031	mean of contrast 4	7.2523	2.1206	0.1546
7	*Pleodorina indica*	5.5527	1.8062	mean of contrast 6	6.9679	2.0253	0.1548
8	*Volvox rousseletii*	8.0212	2.7093	*Volvox globator*	5.8675	2.4082	0.1398
9	*Colemanosphaera charkowiensis*	4.9155	1.5051	*Platydorina caudata*	5.4183	1.5051	0
10	mean of contrast 8	7.7232	2.5843	mean of contrast 9	5.2359	1.5051	0.4339
11	mean of contrast 7	6.6833	1.9294	mean of contrast 10	7.4236	2.3181	0.5250
12	*Oogamochlamys ettlii*	3.8352	1.2041	*Oogamochlamys gigantea*	3.9128	1.8062	7.7577
13	mean of contrast 12	3.8757	1.6021	*Oogamochlamys zimbabwiensis*	3.6221	1.2041	1.5688

Table S5. Phylogenetically independent contrasts between isogamete and microgamete volumes. (See text for descriptions of contrasts.)

	Mean residual $\log_{10}$ gamete volume (μm^3)	
Contrast	Isogamete	Microgamete
Unicellular	0.3055	–0.6278
Colonial/multicellular 1	–0.1520	–2.1551
Colonial/multicellular 2	–0.1049	–1.6486

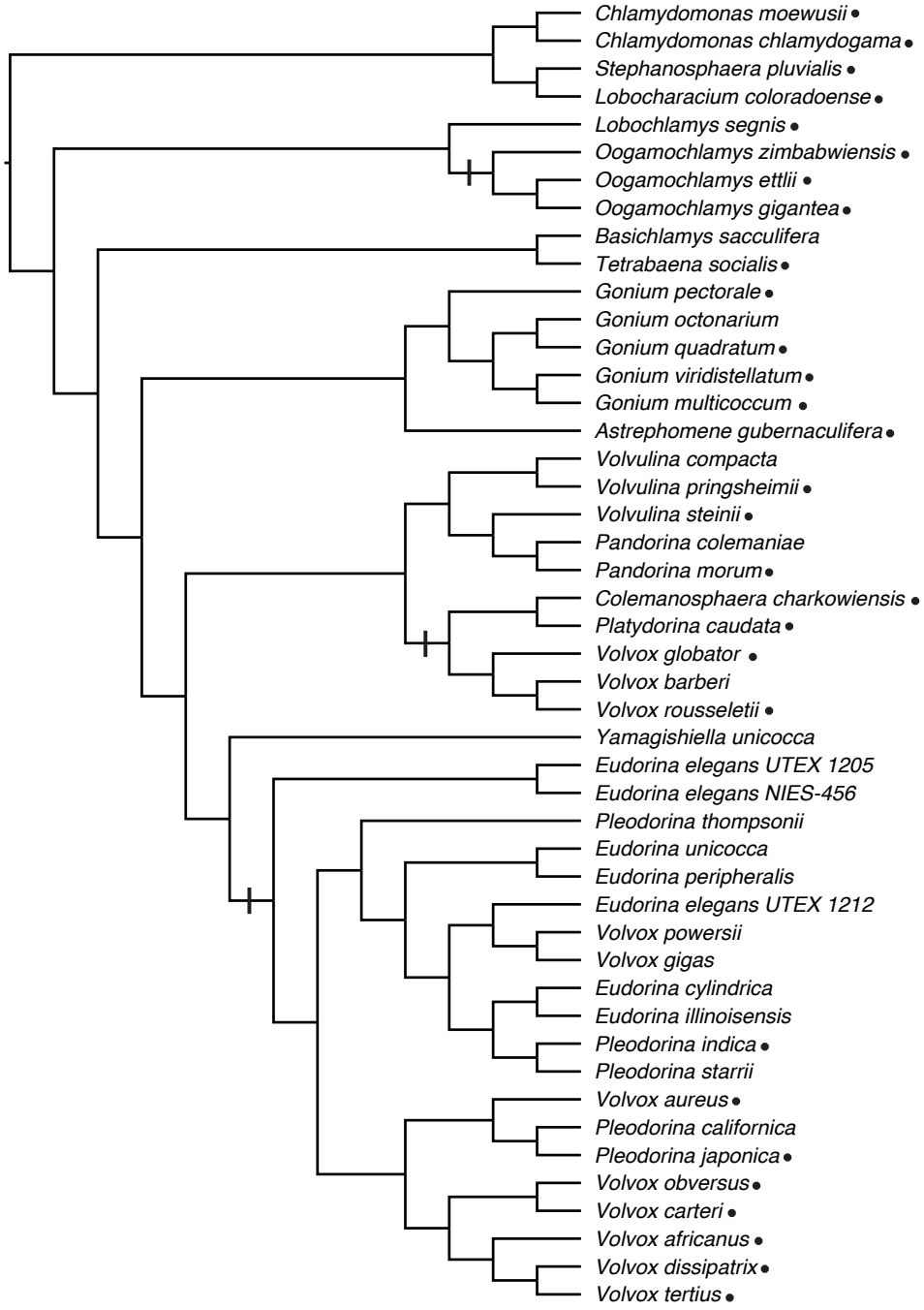

Figure S1. A composite phylogeny of the volvocine algae. Vertical black bars indicate origins of anisogamy (including oogamy). All taxa descending from these origins are anisogamous; all other taxa are isogamous. A dot following a taxon name indicates that gamete and adult volumes are available for the taxon. Details of this phylogeny are given in da Silva [32].

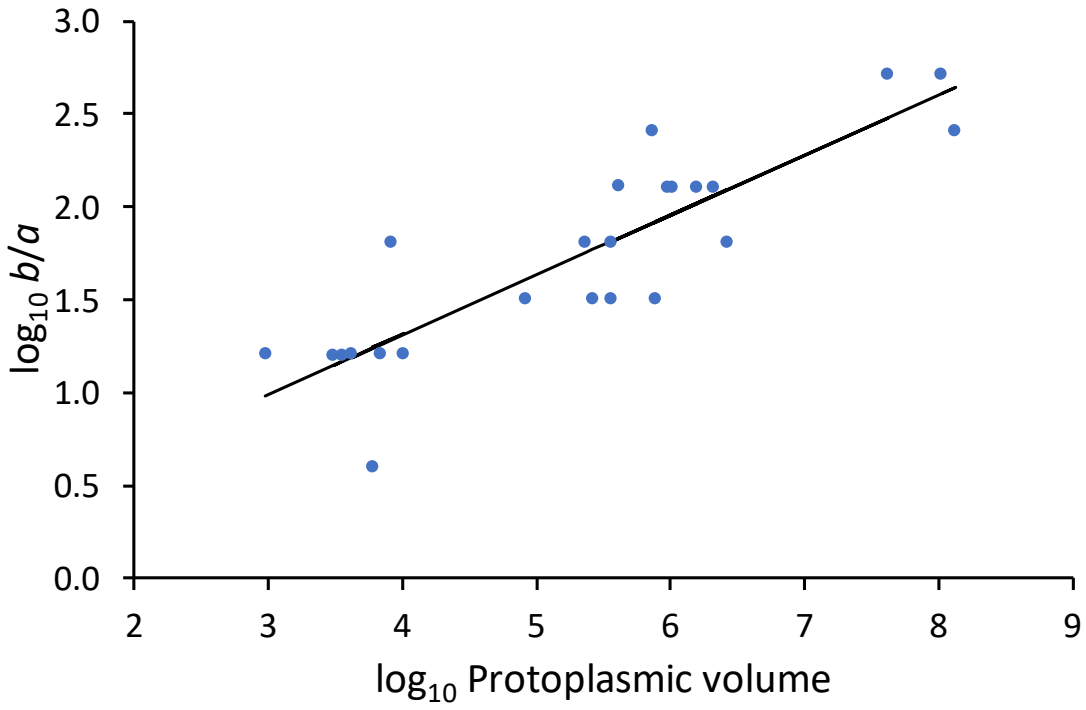

Figure S2. Anisogamy ratio (b/a) regressed against protoplasmic volume for anisogamous species. Regression line: $y = 0.0252 + 0.3220x$; $r^2 = 0.75$; $df = 22, 1$; $F = 65.98$; $P = 4.58 \times 10^{-8}$.

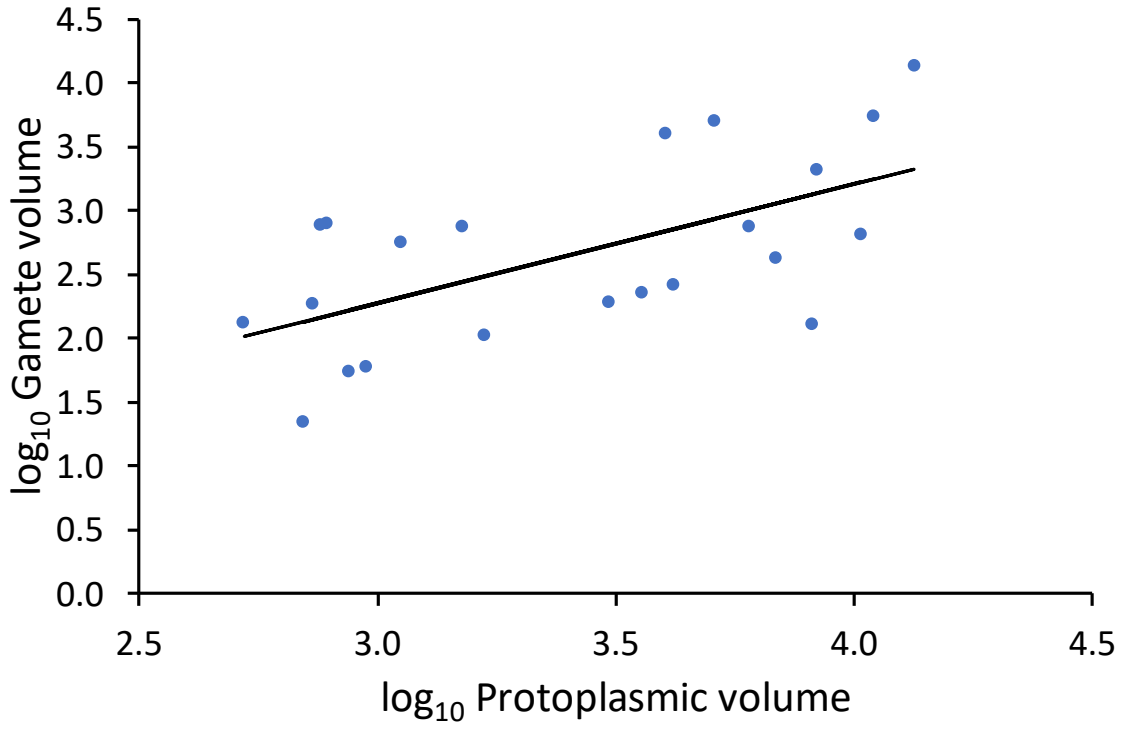

Figure S3. Isogamete and microgamete volumes regressed against protoplasmic volume for unicellular species. Regression line: $y = –0.5280 + 0.9343x$; $r^2 = 0.37$; $df = 1, 20$; $F = 11.69$; $P = 0.0027$.

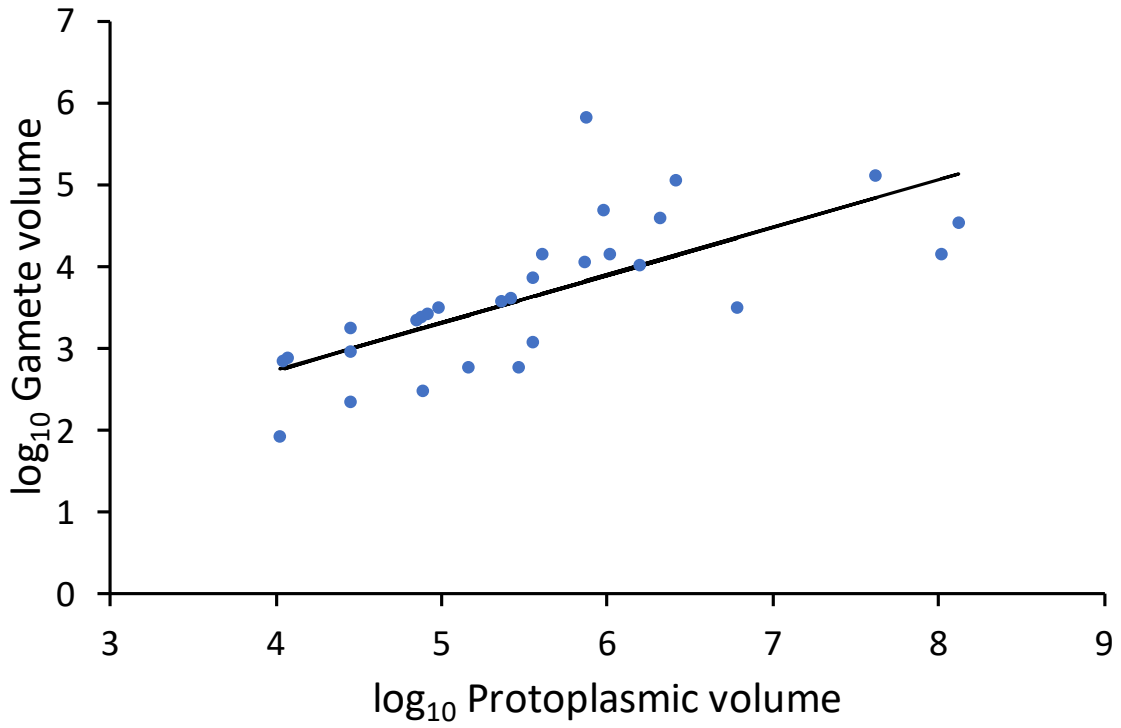

Figure S4. Isogamete and macrogamete volumes regressed against protoplasmic volume for colonial and multicellular species. Regression line: $y = 0.4098 + 0.5807x$; $r^2 = 0.50$; $df = 1, 27$; $F = 26.84$; $P = 1.87 \times 10^{-5}$.

References

1. Kugrens P., Clay B.L., Aguiar R. 2000 Ultrastructure of *Lobocharacium coloradoense*, gen. et sp. nov. (Chlorophyta, Charciciosiphonaceae), an unusual coenocyte from Colorado. *Journal of Phycology* **36**(2), 421-432. (doi:10.1046/j.1529-8817.2000.99089.x).
2. Suda S., Nozaki H., Watanabe M.M. 2005 Morphology and sexual reproduction of *Carteria palmata* sp. nov. belonging to the Carteria group I sensu Lembi (Chlorophyceae, Volvocales). *Phycologia* **44**(6), 596-607. (doi:Doi 10.2216/0031-8884(2005)44[596:Masroc]2.0.Co;2).
3. Bold H.C. 1949 The morphology of *Chlamydomonas chlamydogama*, sp. nov. *Bulletin of the Torrey Botanical Club* **76**(2), 101-108. (doi:10.2307/2482218).
4. Bischoff H.W. 1959 Some observations on *Chlamydomonas microhalophila* sp. nov. *Biological Bulletin* **117**(1), 54-62. (doi:10.2307/1539037).
5. Triemer R.E., Brown R.M. 1975 The ultrastructure of fertilization in *Chlamydomonas moewusii*. *Protoplasma* **84**(3), 315-325. (doi:10.1007/bf01279360).
6. Ende H.V.D. 1985 Sexual agglutination in chlamydomonads. In *Advances in Microbial Physiology* (eds. Rose A.H., Tempest D.W.), pp. 89-123, Academic Press.
7. Martin N.C., Goodenough U.W. 1975 Gametic differentiation in *Chlamydomonas reinhardtii* I. Production of gametes and their fine-structure. *J Cell Biol* **67**(3), 587-605. (doi:DOI 10.1083/jcb.67.3.587).
8. Deason T.R. 1967 *Chlamydomonas gymnogama*, a new homothallic species with naked gametes. *Journal of Phycology* **3**(2), 109-112. (doi:10.1111/j.1529-8817.1967.tb04642.x).
9. Nováková S. 2005 *Pseudocarteria corcontica*, a new quadriflagellate species (Volvocales). *Preslia* **77**(4), 453-457.
10. Ling H.U. 2001 Snow algae of the Windmill Islands, continental Antarctica: *Desmotetra aureospora*, sp. nov. and *D. antarctica*, comb. nov. (Chlorophyta). *Journal of Phycology* **37**(1), 160-174. (doi:10.1046/j.1529-8817.2001.037001160.x).
11. Stein J.R. 1959 The four-celled species of *Gonium*. *American journal of botany*, 366-371.
12. Nozaki H. 1993 Asexual and sexual reproduction in *Gonium quadratum* (chlorophyta) with a discussion of phylogenetic relationship within the Goniaceae. *Journal of Phycology* **29**(3), 369-376. (doi:10.1111/j.0022-3646.1993.00369.x).
13. Nozaki H. 1989 Morphological variation and reproduction in *Gonium viridistellatum* (Volvocales, Chlorophyta). *Phycologia* **28**(1), 77-88. (doi:doi:10.2216/i0031-8884-28-1-77.1).
14. Nozaki H., Kuroiwa T. 1991 Morphology and sexual reproduction of *Gonium multicoccum* (Volvocales, Chlorophyta) from Nepal. *Phycologia* **30**(5), 381-393. (doi:doi:10.2216/i0031-8884-30-5-381.1).
15. Nakada T., Tomita M., Nozaki H. 2010 *Volvulina compacta* (Volvocaceae, Chlorophyceae), new to Japan, and its phylogenetic position. *Journal of Japanese Botany* **85**(6), 364-369.
16. Nozaki H., Kuroiwa T. 1990 *Volvulina compacta* sp. nov. (Volvocaceae, Chlorophyta) from Nepal. *Phycologia* **29**(4), 410-417. (doi:10.2216/i0031-8884-29-4-410.1).
17. Starr R.C. 1962 A new species of *Volvulina* Playfair. *Archiv für Mikrobiologie* **42**(2), 130-137. (doi:10.1007/bf00408169).

18. Nozaki H., Kuroiwa T. 1992 Ultrastructure of the extracellular matrix and taxonomy of *Eudorina*, *Pleodorina* and *Yamagishiella* gen. nov. (Volvocaceae, Chlorophyta). *Phycologia* **31**(6), 529-541. (doi:doi:10.2216/i0031-8884-31-6-529.1).
19. Rayburn W.R., Starr R.C. 1974 Morphology and nutrition of *Pandorina unicocca* sp. nov. *Journal of Phycology* **10**(1), 42-49. (doi:10.1111/j.1529-8817.1974.tb02676.x).
20. Stein J.R. 1958 A morphological study of *Astrephomene gubernaculifera* and *Volvulina steinii*. *American journal of botany* **45**(5), 388-397. (doi:10.2307/2439639).
21. Jaenicke L., Starr R.C. 1996 The lurlenes, a new class of plastoquinone-related mating pheromones from *Chlamydomonas allensworthii* (Chlorophyceae). *European Journal of Biochemistry* **241**(2), 581-585.
22. Demchenko E., Mikhailiuk T. 2012 Peculiarities of sexual reproduction of some new for the flora of Ukraine and rare species of Volvocales (Chlorophyta). *International Journal on Algae* **14**(2), 120-135.
23. Pröschold T., Marin B., Schlösser U.G., Melkonian M. 2001 Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). I. Emendation of *Chlamydomonas ehrenberg* and *Chloromonas gobi*, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. *Protist* **152**(4), 265-300. (doi:http://dx.doi.org/10.1078/1434-4610-00068).
24. Goldstein M. 1964 Speciation and mating behavior in *Eudorina*. *The Journal of Protozoology* **11**(3), 317-344. (doi:10.1111/j.1550-7408.1964.tb01762.x).
25. Nozaki H., Yamada T., Takahashi F., Matsuzaki R., Nakada T. 2014 New "missing link" genus of the colonial volvocine green algae gives insights into the evolution of oogamy. *BMC Evolutionary Biology* **14**(1), 37.
26. Iyengar M.O.P. 1933 Contributions to our knowledge of the colonial Volvocales of South India. *Journal of the Linnean Society of London, Botany* **49**(329), 323-373. (doi:10.1111/j.1095-8339.1933.tb00391.x).
27. Nozaki H., Ott F.D., Coleman A.W. 2006 Morphology, molecular phylogeny and taxonomy of two new species of *Pleodorina* (Volvoceae, Chlorophyceae). *Journal of Phycology* **42**(5), 1072-1080. (doi:10.1111/j.1529-8817.2006.00255.x).
28. Nozaki H., Kuroiwa H., Mita T., Kuroiwa T. 1989 *Pleodorina japonica* sp. nov. (Volvocales, Chlorophyta) with bacteria-like endosymbionts. *Phycologia* **28**(2), 252-267. (doi:doi:10.2216/i0031-8884-28-2-252.1).
29. Nozaki H., Mahakham W., Athibai S., Yamamoto K., Takusagawa M., Misumi O., Herron M.D., Rosenzweig F., Kawachi M. 2017 Rediscovery of the species of 'ancestral *Volvox*': morphology and phylogenetic position of *Pleodorina sphaerica* (Volvocales, Chlorophyceae) from Thailand. *Phycologia* **56**(4), 469-475. (doi:10.2216/17-3.1).
30. Pringsheim E.G. 1970 Identification and cultivation of European *Volvox* spp. *Anton Van Lee J M S* **36**(1), 33-43. (doi:Doi 10.1007/Bf02069005).
31. Kuroiwa H., Nozaki H., Kuroiwa T. 1993 Preferential digestion of chloroplast nuclei in sperms before and during fertilization in *Volvox carteri*. *Cytologia* **58**(3), 281-291. (doi:10.1508/cytologia.58.281).
32. da Silva J. 2018 The evolution of sexes: A specific test of the disruptive selection theory. *Ecology and Evolution* **8**(1), 207-219. (doi:10.1002/ece3.3656).
33. Rosowski J.R., Hoshaw R.W. 1988 Advanced anisogamy in *Chlamydomonas monadina* (Chlorophyceae) with special reference to vacuolar activity during sexuality. *Phycologia* **27**(4), 494-504. (doi:10.2216/i0031-8884-27-4-494.1).

34. Pollio A., Cennamo P., Ciniglia C., De Stefano M., Pinto G., A.R. Huss V. 2005 *Chlamydomonas pitschmannii* Ettl, a little known species from thermoacidic environments. *Protist* **156**(3), 287-302. (doi:https://doi.org/10.1016/j.protis.2005.04.004).
35. Mitra A.K. 1950 A peculiar method of sexual reproduction in certain new members of the Chlamydomonadaceae. *Hydrobiologia* **2**(3), 209-216. (doi:10.1007/BF00046556).