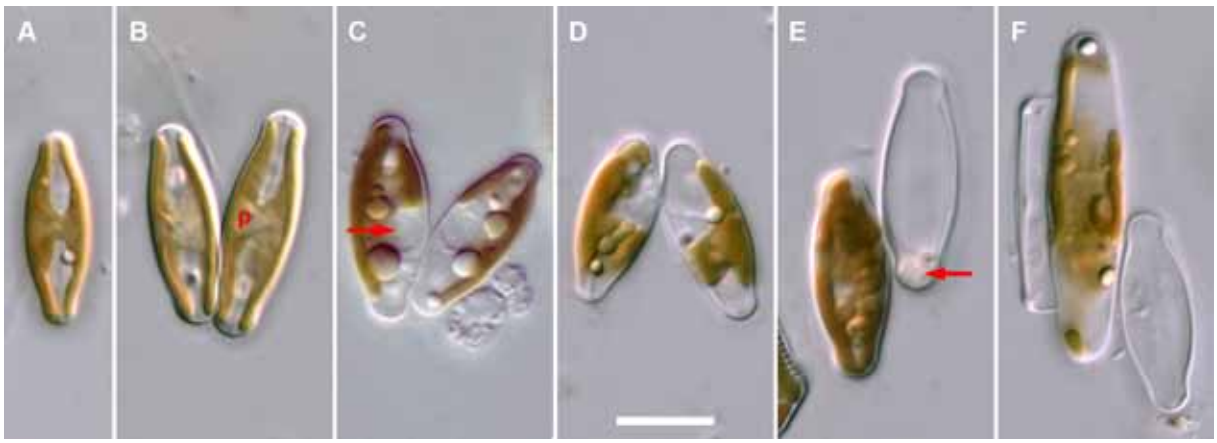

Fig. S1

Fig. S1. The sexual process in *Sellaphora* cf. *auldreekie* in natural populations from Blackford Pond and Lochend Loch, Edinburgh. The species shown is probably *S. auldreekie* sensu stricto in all except in (B) and (E), which are probably of 'Czech auldreekie'. As in other *Sellaphora*, after pairing, one functional gamete was formed per gametangium and as a result one auxospore was formed per pair of gametangia. The gamete produced by one gametangium was active ('male'), while that produced by the other was passive ('female'); consequently, the auxospore was always formed within the female gametangium containing the passive gamete. (A) Unsexualized vegetative cell with a single H-shaped chloroplast. (B) Paired cells, before chloroplast rearrangement; note tetrahedral pyrenoid (p). (C) Pair during meiotic prophase, with enlarged nuclei (e.g. arrow) and rearranged chloroplast. (D) Pair in final stages of gametogenesis, after meiosis II. (E) After completion of plasmogamy: the zygote lies entirely within the female gametangium (left); within the male gametangium there is a small residual body (arrow), containing material excluded from the male gamete. (F) Expanded auxospore. Scale bar 10 μm.

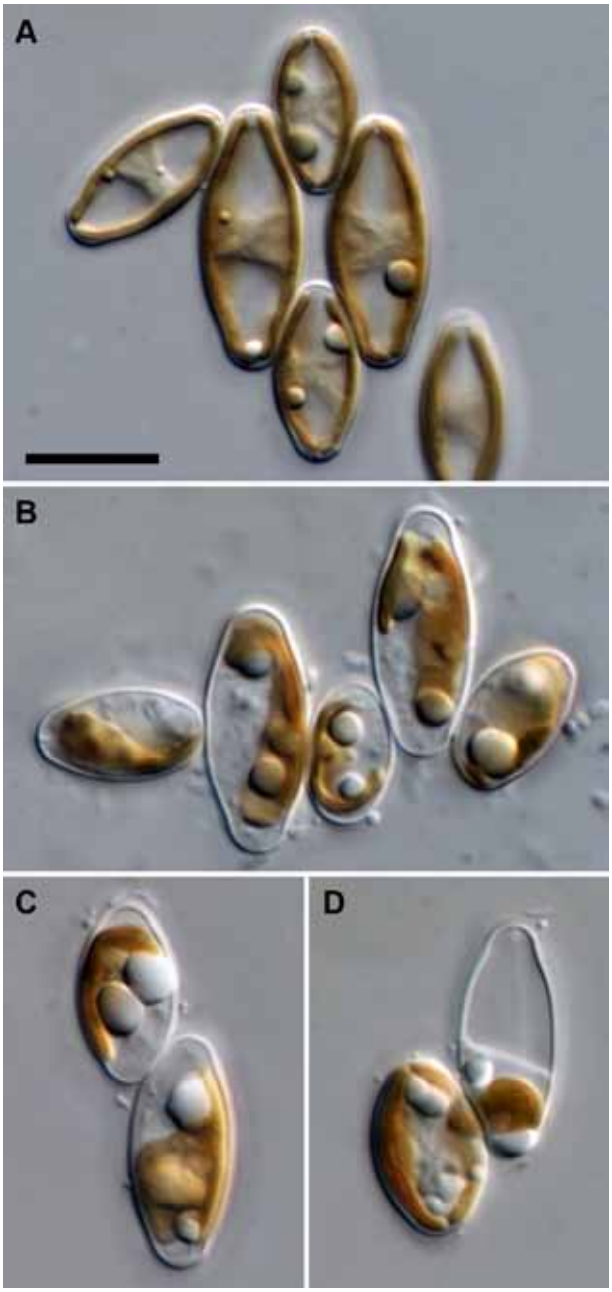

Fig. S2

Fig. S2. Interclonal crosses of *auldreekie*-like *Sellaphora* species. (A) *Sellaphora auldreekie* sensu stricto, Sel775INV (larger cells) × Cr44: early stage in pairing, before chloroplast rearrangement. The cells of one clone bond only to cells of the other clone, not to each other. (B) *Sellaphora* 'coarse auldreekie', SEL620K (larger) × Bolin A10: late gametogenesis, during or after meiosis II. Note the greater widths of the valve poles relative to (A) or (C). (C and D) *Sellaphora* 'southern auldreekie', SEL629K (larger) × Kew A6 in late gametogenesis (C) and during plasmogamy (D). Scale bar 10 μm.

Table S1. Source localities from which *Sellaphora* strains used in this study were isolated.

Source locality	Latitude and longitude
Bolin Bolin Billabong, Melbourne, Australia	37° 46′ 09″S, 145° 04′ 42″E
Croydon Library Pond, Melbourne, Australia	37° 48′ 01″S, 145° 16′ 55″E
Kew Billabong, Melbourne, Australia	37° 47′ 17″S, 145° 02′ 36″E
Streeton Pond, Melbourne, Australia	37° 43′ 43″S, 145° 05′ 40″E
Lake Mumblin, Victoria, Australia	38° 19′ 08″S, 142° 54′ 52″E
Černý Pond, Czech Republic	50° 36’ 31” N, 14° 45’ 46” E
Blackford Pond, Edinburgh, UK	55° 55′ 29″N, 3° 11′ 49″W
Dunsapie Loch, Edinburgh, UK	55° 56′ 43″N, 3° 09′ 11.5″W
Inverleith Pond, Edinburgh, UK	55° 57′ 41″N, 3° 04′ 04.5″W
Lochend Loch, Edinburgh, UK	55° 57′ 38″N, 3° 09′ 42.5″W

Table S2. *Sellaphora* strain information. Strains indicated in gray were used in the crossing experiments 2008-2009; n/a not applicable, n/d no data. Accession numbers in bold are new to this study, others were published in Hamsher et al. (2011).

Designation	Strain	Origin	Collection date	Collector/ isolator	Voucher slide	GenBank Accession *cox*1	GenBank Accession *rbc*L	Valve length at first cross (µm)
S. auldreekie	Sel492D	Dunsapie Loch, UK	12 Oct. 2004	D.G. Mann/ A. Poulíčková	E5185/5255	n/d	HQ317110 and HQ337587	12.3 ± 0.7
S. auldreekie	Cr44	Croydon Library Pond, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E3772	KC911817	KC911803	14.9 ± 0.6
S. auldreekie	Sel776INV	Inverleith Pond, UK	3 Mar. 2008	D.G. Mann/ D.G. Mann	E4289	KC911818	KC911804	20.8 ± 0.4
S. auldreekie	Sel781INV	Inverleith Pond, UK	3 Mar. 2008	D.G. Mann/ D.G. Mann	E4290	KC911819	KC911805	20.9 ± 0.4
S. auldreekie	Sel775INV	Inverleith Pond, UK	3 Mar. 2008	D.G. Mann/ D.G. Mann	E4288	KC911820	n/d	20.6 ± 0.4
S. pupula agg. 'southern auldreekie'	Sel630K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4164	KC911822	n/d	18.6 ± 0.3
S. pupula agg. 'southern auldreekie'	KewA3	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ P. Vanormelingen	E4244	KC911823	KC911806	11.9 ± 0.7
S. pupula agg. 'southern auldreekie'	Sel629K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4163	KC911824	KC911807	18.7 ± 0.3
S. pupula agg. 'southern auldreekie'	Sel624K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4158	KC911821	n/d	16.8 ± 0.4
S. pupula agg. 'southern auldreekie'	Str06	Streeton Pond, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E3784	KC911825	n/d	16.3 ± 0.7
S. pupula agg. 'southern auldreekie'	KewA6	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ P. Vanormelingen	E4243	KC911826	n/d	14.2 ± 0.6
S. pupula agg. 'southern auldreekie'	Kew21	Kew Billabong, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E3785	HQ317094	HQ317119 and HQ337598	19.5 ± 0.5

S. pupula agg. 'southern auldreekie'	Sel635K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4167	KC911827	n/d	17.1 ± 0.4
S. pupula agg. 'southern auldreekie'	Sel618K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4152	KC911828	n/d	17.0 ± 0.2
S. pupula agg. 'southern auldreekie'	Kew27	Kew Billabong, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E3780	KC911829	n/d	n/a
S. pupula agg. 'southern auldreekie'	KewA7	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ P. Vanormelingen	E4248	KC911830	n/d	n/a
S. pupula agg. 'southern auldreekie'	BB83	Bolin Bolin Billabong, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E4378	KC911831	n/d	n/a
S. pupula agg. 'coarse auldreekie'	BolinA2	Bolin Bolin Billabong, Australia	Dec. 2007	K.M. Evans/ P. Vanormelingen	E4247	KC911832	KC911808	10.4 ± 0.8
S. pupula agg. 'coarse auldreekie'	BolinA4	Bolin Bolin Billabong, Australia	Dec. 2007	K.M. Evans/ P. Vanormelingen	E4245	KC911833	n/d	10.4 ± 0.7
S. pupula agg. 'coarse auldreekie'	Sel620K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4154	KC911834	n/d	19.3 ± 0.3
S. pupula agg. 'coarse auldreekie'	Sel686K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4181	n/d	n/d	17.4 ± 0.5
S. pupula agg. 'coarse auldreekie'	Sel642K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4174	HQ317107	HQ337611 and KC911809	22.1 ± 0.5
S. pupula agg. 'coarse auldreekie'	Sel627K	Kew Billabong, Australia	2 Dec. 2007	K.M. Evans/ D.G. Mann	E4161	KC911835	KC911810	21.0 ± 0.4
S. pupula agg. 'coarse auldreekie'	BolinA10	Bolin Bolin Billabong, Australia	Dec. 2007	K.M. Evans/ P. Vanormelingen	E4246	KC911836	n/d	12.8 ± 0.3
S. pupula agg. "another auldreekie"	Str13	Streeton Pond, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E3762	n/d	KC911811	n/a

S. pupula agg. "another auldreekie"	BB94	Bolin Bolin Billabong, Australia	Jan. 2007	K.M. Evans/ V.A. Chepurnov	E4379	HQ317096	HQ317121 and HQ337600	n/a
S. pupula agg. 'Czech auldreekie'	LE J9	Lochend Loch, UK	May 2007	D.G. Mann/ D.G. Mann	E5253	KC911837	KC911812	13.0 ± 0.9
S. pupula agg. 'Czech auldreekie'	LE D35	Lochend Loch, UK	May 2007	D.G. Mann/ D.G. Mann	E3823	KC911838	KC911813	15.9 ± 1.1
S. pupula agg. 'Czech auldreekie'	28/28	Černý Pond	May 2007	A. Poulíčková/ A. Poulíčková	E4353	HQ317105	HQ317129 and HQ337609	n/a
S. pupula agg. 'mumblin auldreekie'	mm111	Lake Mumblin, Australia	27 Nov. 2007	K.M. Evans/ V.A. Chepurnov	E4469	HQ317106	HQ337610	n/a
S. pupula agg. 'mumblin auldreekie'	mm120	Lake Mumblin, Australia	27 Nov. 2007	K.M. Evans/ V.A. Chepurnov	E4411	KC911839	n/d	n/a
S. pupula agg. 'mumblin auldreekie'	mm135	Lake Mumblin, Australia	27 Nov. 2007	K.M. Evans/ V.A. Chepurnov	E4418	KC911840	KC911814	n/a
S. pupula agg. 'mumblin auldreekie'	mm178	Lake Mumblin, Australia	27 Nov. 2007	K.M. Evans/ V.A. Chepurnov	E4322	n/d	KC911815	n/a
S. pupula agg. 'mumblin auldreekie'	mm185	Lake Mumblin, Australia	27 Nov. 2007	K.M. Evans/ V.A. Chepurnov	E4321	KC911841	KC911816	n/a

Table S3. Results of crosses made on 31 October 1996 between strains of *Sellaphora auldreekie* from Blackford Pond (clones with –B suffix) and Dunsapie Loch (clones with –D suffix), Edinburgh. When isolated, strains had lengths varying from 16–22.5 µm and all were able to reproduce sexually when mixed with a compatible partner. When the gametangia of the two mating types could be distinguished from each other (because they differed in size), it could be seen that all the gametangia produced by one mating type were female and all those produced by the other mating type were male. Intraclonal auxosporulation occurred only in two strains (the female strains 3B and 22B) and only infrequently in these, relative to the abundance of auxosporulation in crosses between compatible strains. Initial cells formed in crosses between strains 7B and 20B made in December 1996, two months after isolation of the strains, were 28.5–33 µm long (31.0 ± 1.17, mean ± S.D., n = 40). The largest-celled strain tested (16D) which was > 22 µm long, did not become sexualized in any cross and may have been above the critical size threshold for sexualization. Crosses were scored on 5 November 1996, as follows: + cross successful (**bold** indicates that the direction of gamete movement, and hence the sexes of the clones could be determined because the clones could be told apart by cell length), – cross unsuccessful, n/d no data, [0] no auxosporulation in monoclonal culture, [T] infrequent auxosporulation in monoclonal culture.

Clone	Sex	1B	4B	6B	8B	11B	12B	14B	20B	3D	3B	7B	17B	22B	17D	21D
1B	Sau$^+$	[0]														
4B	Sau$^+$	0	[0]													
6B	Sau$^+$	0	0	[0]												
8B	Sau$^+$	0	0	0	[0]											
11B	Sau$^+$	0	0	0	0	[0]										
12B	Sau$^+$	0	0	0	0	0	[0]									
14B	Sau$^+$	0	0	0	0	0	0	[0]								
20B	Sau$^+$	0	0	0	0	0	0	0	[0]							
3D	Sau$^+$	0	0	0	n/d	n/d	n/d	n/d	0	[0]						
3B	Sau$^-$	+	+	**+**	**+**	**+**	**+**	**+**	**+**	+	[T]					
7B	Sau$^-$	**+**	**+**	**+**	**+**	**+**	+	**+**	**+**	**+**	0	[0]				
17B	Sau$^-$	**+**	**+**	**+**	+	+	**+**	**+**	**+**	n/d	0	0	[0]			
22B	Sau$^-$	+	**+**	**+**	**+**	+	**+**	**+**	+	n/d	0	0	0	[T]		
17D	Sau$^-$	n/d	n/d	**+**	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0	n/d	n/d	[0]	
21D	Sau$^-$	n/d	n/d	**+**	n/d	n/d	**+**	n/d	n/d	n/d	n/d	0	0	n/d	0	[0]

Table S4. Valve length, midwidth, pole width, and striation density of auldreekie-like *Sellaphora* strains used in this study. Values are mean ± standard deviation (range), n = 10 per strain. The column 'subset' indicates the belonging of the strain to the different size groupings made for the *a posteriori* morphological analysis.

Strain	Species	Length (μm)	Subset	Pole width (μm)	Stria density (# in 10 μm)
F1(776-775)1	*S. auldreekie*	25.9 ± 0.2 (25.5-26.3)	F1	3.97 ± 0.12 (3.77-4.20)	22.5 ± 0.7 (21.3-23.8)
F1(776-775)2	*S. auldreekie*	26.4 ± 0.4 (25.8-27.0)	F1	3.97 ± 0.09 (3.85-4.14)	22.6 ± 0.7 (21.5-23.8)
F1(776-775)3	*S. auldreekie*	23.9 ± 0.3 (23.5-24.6)	F1	3.90 ± 0.10 (3.77-4.12)	22.6 ± 0.6 (21.7-23.4)
Sel492D	*S. auldreekie*	20.2 ± 0.2 (19.7-20.5)	Medium	3.73 ± 0.08 (3.62-3.91)	22.7 ± 0.7 (21.1-23.7)
Sel492D	*S. auldreekie*	12.0 ± 0.6 (10.9-12.7)	Small	3.76 ± 0.12 (3.58-3.95)	22.2 ± 0.8 (20.5-23.7)
Cr44	*S. auldreekie*	16.2 ± 0.7 (15.5-18.0)	Medium	3.59 ± 0.07 (3.48-3.68)	22.7 ± 0.5 (22.1-23.9)
Sel775INV	*S. auldreekie*	19.5 ± 0.2 (19.2-19.7)	Medium	3.77 ± 0.07 (3.70-3.93)	22.8 ± 0.7 (21.3-23.6)
F1(629-Kew21)1	*S. pupula* agg. 'southern auldreekie'	27.4 ± 0.4 (26.8-28.0)	F1	3.81 ± 0.16 (3.56-4.09)	23.2 ± 0.6 (22.5-24.1)
F1(629-Kew21)2	*S. pupula* agg. 'southern auldreekie'	26.9 ± 0.4 (26.3-27.7)	F1	3.84 ± 0.10 (3.68-4.05)	23.5 ± 0.7 (22.3-24.3)
F1(629-Kew21)3	*S. pupula* agg. 'southern auldreekie'	27.5 ± 0.3 (27.1-27.9)	F1	3.99 ± 0.06 (3.89-4.07)	23.4 ± 0.7 (22.5-24.3)
Kew A3	*S. pupula* agg. 'southern auldreekie'	12.0 ± 0.8 (10.4-13.1)	Small	3.55 ± 0.10 (3.37-3.72)	23.6 ± 0.9 (22.1-24.9)
Str06	*S. pupula* agg. 'southern auldreekie'	18.5 ± 0.5 (17.9-19.3)	Medium	3.34 ± 0.18 (3.09-3.60)	24.1 ± 0.6 (23.4-25.3)
Kew 21	*S. pupula* agg. 'southern auldreekie'	20.5 ± 0.3 (20.1-21.1)	Medium	3.79 ± 0.06 (3.70-3.87)	23.3 ± 0.7 (22.3-24.5)
BB83	*S. pupula* agg. 'southern auldreekie'	10.9 ± 0.9 (9.8-12.4)	Small	3.53 ± 0.18 (3.27-3.79)	23.3 ± 0.7 (22.3-24.3)
F1(620-642)1	*S. pupula* agg. 'coarse auldreekie'	28.7 ± 0.4 (28.1-29.3)	F1	4.30 ± 0.09 (4.16-4.47)	22.6 ± 1.0 (20.1-23.4)
F1(620-642)1	*S. pupula* agg. 'coarse auldreekie'	28.8 ± 0.4 (28.1-29.3)	F1	4.40 ± 0.08 (4.26-4.49)	22.4 ± 0.6 (21.5-23.4)
F1(620-642)1	*S. pupula* agg. 'coarse auldreekie'	28.6 ± 0.3 (28.0-28.9)	F1	4.27 ± 0.11 (4.07-4.47)	22.9 ± 0.9 (21.9-24.1)
Bolin A4	*S. pupula* agg. 'coarse auldreekie'	10.6 ± 0.8 (9.7-11.9)	Small	3.84 ± 0.09 (3.64-3.91)	23.5 ± 1.1 (22.3-26.0)
Sel620K	*S. pupula* agg. 'coarse auldreekie'	19.4 ± 0.3 (18.8-19.8)	Medium	4.05 ± 0.04 (4.00-4.11)	22.6 ± 1.0 (20.7-24.5)
Sel642K	*S. pupula* agg. 'coarse auldreekie'	22.6 ± 0.3 (22.1-23.0)	Medium	4.18 ± 0.04 (4.12-4.25)	22.9 ± 0.6 (21.7-23.9)
Sel627K	*S. pupula* agg. 'coarse auldreekie'	21.2 ± 0.2 (20.9-21.4)	Medium	4.13 ± 0.05 (4.06-4.23)	22.2 ± 0.7 (21.2-23.2)
Str13	*S. pupula* agg. "another auldreekie"	19.2 ± 0.3 (18.7-19.8)	Medium	3.81 ± 0.06 (3.74-3.87)	23.2 ± 0.8 (22.1-25.0)
BB94	*S. pupula* agg. "another auldreekie"	10.9 ± 0.8 (9.2-11.8)	Small	4.01 ± 0.13 (3.83-4.21)	22.8 ± 0.7 (22.1-24.7)
LE J9	*S. pupula* agg. 'end auldreekie'	12.4 ± 0.8 (11.1-13.3)	Small	3.77 ± 0.14 (3.58-3.97)	22.6 ± 0.9 (21.4-23.7)
LE D35	*S. pupula* agg. 'end auldreekie'	22.8 ± 0.2 (22.4-23.2)	Medium	4.19 ± 0.07 (4.09-4.34)	21.8± 0.9 (20.7-23.4)
mm111	*S. pupula* agg. 'mumblin auldreekie'	20.2 ± 0.5 (19.3-21.1)	Medium	4.05 ± 0.10 (3.85-4.19)	22.4 ± 0.8 (21.5-23.8)
mm120	*S. pupula* agg. 'mumblin auldreekie'	15.8 ± 0.6 (15.0-16.6)	Medium	4.13 ± 0.06 (4.07-4.26)	22.1 ± 1.1 (20.8-23.8)