
Supplementary Material 3:

Detailed discussion regarding the classification putative hybrids, and detailed sample allocation data

Associated paper: Assessing a Bayesian approach for detecting exotic hybrids between plantation and native eucalypts

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Of the 15 putative hybrids 12 were classified as hybrids and three could not be distinguished from their pure parents in the two-way analysis. Of the three indistinguishable samples, the two putative *E. cypellocarpa* F₁s are probably correctly classified as pure *E. cypellocarpa*. These two samples were collected from seedlings growing among native *E. cypellocarpa* beside a 10 year old *E. globulus* plantation in Gippsland in the absence of native *E. globulus* – ruling out the possibility that they are backcrosses (i.e. no mature F₁s available to facilitate backcrossing). As noted previously the seedling morphology of these two species is very similar and can be difficult to distinguish, and these two samples were only tentatively classified as “possible” F₁s (with a low degree of certainty) based on the degree of glaucousness, which can be a highly variable trait, even within species [69]. Therefore given that they overlap the 95% confidence intervals of simulated pure *E. cypellocarpa*, and there was low confidence in the morphological assessment, it is likely that they are correctly allocated as pure *E. cypellocarpa*.

The situation with the putative *E. viminalis* x *globulus* hybrid that was classified as *E. globulus* is less clear. This sample was identified in open pollinated *E. globulus* seed, collected from native forest in southern Tasmania, where the species co-occur in the absence of other cross-compatible eucalypts in close proximity. The sample showed distinctively intermediate morphology, with linear, non-glaucous leaves compared to the broadly-linear, glaucous leaves of *E. globulus* and the narrowly-linear non- glaucous leaves of *E.viminalis*. The sample could be a morphologically unusual pure *E. globulus* that is coincidentally intermediate between the species. However, the likelihood of random morphological deviations on multiple traits resulting in intermediate characteristics is low, and is more easily

explained by inter-specific hybridisation [70, 71]. Therefore, considering the model inaccuracy when identifying backcrosses, the native forest setting of the mother, and the morphology, it is possible that this sample is actually a first or perhaps later generation backcross.

Of the four putative *E. camaldulensis* x *globulus* samples collected from open pollinated seed, three were within the 95% confidence intervals of the mean of the simulated F_1 's, providing good evidence they are F_1 hybrids between *E. camaldulensis* and *E. globulus* Table 1. This supports Barbour *et al.* [30] who also identified putative *E. camaldulensis* x *globulus* F_1 s in seedlots from the same region based on morphology. The fourth sample from this seedlot had mean q values outside the 95% confidence intervals of the simulated F_1 s, but its 95% confidence intervals did include the mean of the simulated F_1 s (Table 1). These four samples were classified as putative F_1 s because the age of plantation neighbouring the mothers (10 years) made it unlikely that a mature F_1 hybrid could occur to facilitate backcrossing. Moreover, the q values of the fourth sample resemble a backcross to *E. globulus* (Table 1), which is impossible given that it came from an *E. camaldulensis* seedlot. The sample could be a hybrid with another species, although the six-way analysis shows that none of the other species in the model were involved, despite *E. ovata* and *E. viminalis* occurring in the area where the seed was collected. Therefore given the somewhat divergent nature of this sample it could be an exotic F_1 , or it could be a hybrid with an unknown species.

The putative *E. viminalis* x *globulus* hybrids (generation unknown) were mature trees identified on the basis of intermediate capsule morphology within native forests, and were classified as hybrids in the two-way model. Both natural and manipulated hybridisation between the species has been reported [56]. All three samples here have q values that are more consistent with backcrosses towards *E. globulus* than F_1 s or F_2 s (Table 5). They were collected from native forests that were dominated by *E. globulus* with scattered *E. viminalis*. This demographic ratio might be conducive to the formation of hybrids due to pollen swamping by the more numerous species [72], and in such a situation backcross hybrids towards *E. globulus* would be common. Patterns of asymmetrical gene flow in native eucalypts have also arisen through differences in flower size [73]. It has been shown that in many cases the pollen tube of small flowered species cannot reach the ovaries of large flowered species, while the reverse cross can work [74]. *Eucalyptus globulus* has

significantly larger flowers than *E. viminalis* [69] again making backcrosses towards *E. globulus* more likely. Therefore these samples are most likely backcrosses to *E. globulus* as suggested by the admixture analysis, rather than F₁ or F₂ hybrids.

The five putative *E. cypellocarpa* x *globulus* hybrids (generation unknown) have *q* values that are consistent with F₁s/F₂s or backcrosses towards *E. cypellocarpa* (Table 2). The sample from a mature tree in native forest at Moonlight Head has *q* values that fall within the 95% confidence intervals of the simulated F₁s. It was found in forest where both species were common, and was unique among the trees inspected in that it had intermediate capsule morphology. Natural F₁ hybrids between these species have been reported from mixed stands elsewhere in Victoria [38, 75] and the simplest explanation is probably that this sample is an F₁ that has survived to reproductive maturity.

The hybrid status of the population where the four Mallacoota samples were collected has been the focus of considerable study [38, 39, 76]. All trees in the population appear to be intermediate to varying degrees between *E. cypellocarpa* and *E. globulus*. Despite nearby sources of *E. cypellocarpa* the nearest *E. globulus* is 6.4 km away. This led to the population being one of the first reported examples of a phantom hybrid zone [38, 39]. There are several other well documented examples of hybrid swarms between the two species in river valleys to the west of Mallacoota where both species are present [38, 39, 75]. The trees in the Mallacoota population occur basically at sea level and are large, probably ranging in age from tens to hundreds of years old. Morphometric analysis showed that none of the trees could be definitively classified as *E. cypellocarpa* or *E. globulus*, but two trees in particular were very *E. globulus*-like [38]. However, terpene analysis found all trees to be indistinguishable from *E. cypellocarpa* [38]. It was concluded that the population is probably of hybrid origin and represents a genetic remnant of the past distribution of *E. globulus* that was flooded when sea level rose after the last glacial maximum [38]. Although we only analysed four samples from this population, they do appear to fit with the Kirkpatrick *et al.* [38] hypothesis, with one sample being consistent with an F₁ or F₂ (although its 95% CIs did include the simulated backcross means; Table 2) and the other three being more similar to backcrosses towards *E. cypellocarpa* (Table 2).

Table 1. Assignment of simulated, pedigreed and putative hybrid samples between *E. camaldulensis* and *E. globulus* obtained from STRUCTURE using a *q* cut-off of 0.2.

Simulated samples	<i>n</i>	% correctly assigned at <i>q</i> >0.2	<i>E. camaldulensis</i> cluster		<i>E. globulus</i> cluster	
			<i>mean q</i>	95% CI (+ -)	<i>mean q</i>	95% CI (+ -)
Simulated <i>E. camaldulensis</i>	50	98	0.902	(0.910,0.891)	0.098	(0.109,0.086)
Simulated <i>E. globulus</i>	50	100	0.084	(0.093,0.076)	0.916	(0.924,0.907)
Simulated F1	50	100	0.530	(0.560,0.501)	0.470	(0.499,0.440)
Simulated F2	50	90	0.526	(0.573,0.478)	0.474	(0.522,0.427)
Simulated BCc	50	60	0.738	(0.770,0.706)	0.262	(0.294,0.230)
Simulated BCg	50	80	0.305	(0.339,0.270)	0.686	(0.721,0.652)
simulation total	300	88.3				
Pedigreed and putative hybrids	<i>n</i>	assignment at <i>q</i> >0.2	<i>mean q</i>	95% CI (+ -)	<i>mean q</i>	95% CI (+ -)
pedigreed F1	1	hybrid	0.506	(0.247,0.768)	0.495	(0.232,0.753)
pedigreed F1	1	hybrid	0.580	(0.308,0.853)	0.421	(0.147,0.692)
Putative F1*	1	hybrid	0.335	(0.108,0.587)	0.665	(0.413,0.892)
Putative F1	1	hybrid	0.536	(0.271,0.799)	0.464	(0.201,0.729)
Putative F1	1	hybrid	0.583	(0.303,0.854)	0.417	(0.146,0.697)
Putative F1	1	hybrid	0.416	(0.171,0.685)	0.584	(0.315,0.829)

* Divergent sample referred to in the text

Table 2. Assignment of simulated, pedigreed and putative hybrid samples between *E. cypellocarpa* and *E. globulus* obtained from STRUCTURE using a *q* cut-off of 0.2. . Putative hybrid[?] = putative hybrid samples collected from mature trees in native forest where the generation is unknown.

Simulated samples	<i>n</i>	% correctly assigned at <i>q</i> >0.2	<i>E. cypellocarpa</i> cluster		<i>E. globulus</i> cluster	
			<i>mean q</i>	95% CI (+ -)	<i>mean q</i>	95% CI (+ -)
Simulated <i>E. cypellocarpa</i>	50	70	0.815	(0.842,0.789)	0.185	(0.211,0.158)
Simulated <i>E. globulus</i>	50	72	0.165	(0.184,0.148)	0.834	(0.852,0.816)
Simulated F1	50	98	0.490	(0.524,0.456)	0.510	(0.544,0.476)
Simulated F2	50	100	0.488	(0.526,0.450)	0.512	(0.550,0.474)
Simulated BCc	50	80	0.677	(0.714,0.639)	0.323	(0.361,0.286)
Simulated BCg	50	86	0.339	(0.378,0.299)	0.648	(0.687,0.609)
Total	300	84.4				
Pedigreed and putative hybrids	<i>n</i>	assignment at <i>q</i> >0.2	<i>E. cypellocarpa</i> cluster		<i>E. globulus</i> cluster	
			<i>mean q</i>	95% CI (+ -)	<i>mean q</i>	95% CI (+ -)
putative hybrid [?]	1	hybrid	0.563	(0.155,0.940)	0.437	(0.060,0.845)
putative hybrid [?] *	1	hybrid	0.669	(0.322,0.960)	0.331	(0.040,0.678)
putative hybrid [?] *	1	hybrid	0.628	(0.278,0.941)	0.372	(0.059,0.722)
putative hybrid [?] *	1	hybrid	0.726	(0.347,0.983)	0.274	(0.017,0.653)
putative hybrid [?] *	1	hybrid	0.494	(0.127,0.868)	0.506	(0.132,0.873)
putative F1	1	<i>E. cypellocarpa</i>	0.864	(0.616,0.996)	0.136	(0.004,0.384)
putative F1	1	<i>E. cypellocarpa</i>	0.848	(0.568,0.995)	0.152	(0.005,0.432)

* Samples from the putative phantom hybrid zone at Mallacoota discussed in the text

Table 3. Assignment of simulated, pedigreed and putative hybrid samples between *E. nitens* and *E. globulus* obtained from STRUCTURE using a q cut-off of 0.2.

Simulated samples	n	% correctly assigned at $q > 0.2$	<i>E. nitens</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
Simulated <i>E. nitens</i>	50	98	0.917	(0.927,0.907)	0.083	(0.093,0.073)
Simulated <i>E. globulus</i>	50	98	0.086	(0.096,0.075)	0.914	(0.925,0.904)
Simulated F1	50	100	0.504	(0.527,0.482)	0.496	(0.518,0.473)
Simulated F2	50	88	0.520	(0.567,0.474)	0.480	(0.526,0.433)
Simulated BCn	50	66	0.728	(0.760,0.696)	0.272	(0.304,0.240)
Simulated BCg	50	68	0.256	(0.289,0.223)	0.730	(0.763,0.698)
Total	300	86.3				
Pedigreed hybrid samples	n	assignment at $q > 0.2$	<i>E. nitens</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
pedigreed F1	1	hybrid	0.6562	(0.408,0.869)	0.3438	(0.131,0.592)
pedigreed F1	1	hybrid	0.442	(0.153,0.721)	0.558	(0.279,0.847)
pedigreed F1	1	hybrid	0.5108	(0.267,0.743)	0.4892	(0.257,0.733)
pedigreed F1	1	hybrid	0.5187	(0.274,0.753)	0.4813	(0.247,0.726)
pedigreed F1	1	hybrid	0.4818	(0.191,0.764)	0.5182	(0.236,0.809)
pedigreed F1	1	hybrid	0.3872	(0.150,0.645)	0.6128	(0.355,0.850)
pedigreed F1	1	<i>E. globulus</i>	0.1659	(0.011,0.402)	0.8341	(0.598,0.989)
pedigreed F1	1	hybrid	0.4274	(0.169,0.700)	0.5726	(0.300,0.831)
pedigreed F1	1	hybrid	0.4327	(0.164,0.694)	0.5673	(0.306,0.836)
pedigreed F1	1	hybrid	0.2366	(0.041,0.491)	0.7634	(0.509,0.959)
pedigreed F1	1	hybrid	0.4423	(0.178,0.711)	0.5577	(0.289,0.822)
pedigreed F1	1	hybrid	0.427	(0.175,0.688)	0.573	(0.312,0.825)
pedigreed F2	1	hybrid	0.6063	(0.353,0.833)	0.3937	(0.167,0.647)
pedigreed F2	1	hybrid	0.4833	(0.218,0.736)	0.5167	(0.264,0.782)
pedigreed F2	1	hybrid	0.5528	(0.271,0.814)	0.4472	(0.186,0.729)
pedigreed F2	1	hybrid	0.3944	(0.137,0.659)	0.6056	(0.341,0.863)
pedigreed BCg	1	<i>E. globulus</i>	0.1713	(0.012,0.404)	0.8287	(0.596,0.988)
pedigreed BCg	1	<i>E. globulus</i>	0.1615	(0.011,0.388)	0.8385	(0.612,0.989)
pedigreed BCg	1	<i>E. globulus</i>	0.1613	(0.018,0.383)	0.8387	(0.617,0.982)
pedigreed BCg	1	<i>E. globulus</i>	0.1434	(0.009,0.346)	0.8566	(0.654,0.991)
pedigreed BCg	1	<i>E. globulus</i>	0.185	(0.022,0.406)	0.815	(0.594,0.978)
pedigreed BCg	1	hybrid	0.2244	(0.028,0.466)	0.7756	(0.534,0.972)
pedigreed BCg	1	hybrid	0.2247	(0.051,0.435)	0.7753	(0.565,0.949)
pedigreed BCg	1	hybrid	0.2204	(0.038,0.445)	0.7796	(0.555,0.962)
pedigreed BCg	1	hybrid	0.2713	(0.062,0.527)	0.7287	(0.473,0.938)
pedigreed BCg	1	<i>E. globulus</i>	0.1317	(0.004,0.356)	0.8683	(0.644,0.996)
pedigreed BCg	1	<i>E. globulus</i>	0.1866	(0.028,0.410)	0.8134	(0.590,0.972)
pedigreed BCg	1	<i>E. globulus</i>	0.115	(0.010,0.284)	0.885	(0.716,0.990)

Table 4. Assignment of simulated, pedigreed and putative hybrid samples between *E. ovata* and *E. globulus* obtained from STRUCTURE using a q cut-off of 0.2.

Simulated samples	n	% correctly assigned at $q > 0.2$	<i>E. ovata</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
Simulated <i>E. ovata</i>	50	100	0.925	(0.933,0.917)	0.075	(0.083,0.067)
Simulated <i>E. globulus</i>	50	98	0.090	(0.102,0.078)	0.910	(0.922,0.898)
Simulated F1	50	94	0.477	(0.514,0.440)	0.523	(0.560,0.486)
Simulated F2	50	98	0.502	(0.544,0.461)	0.498	(0.539,0.456)
Simulated BCo	50	72	0.721	(0.757,0.686)	0.279	(0.314,0.243)
Simulated BCg	50	74	0.278	(0.313,0.242)	0.708	(0.744,0.673)
Total	300	89.3				
Pedigreed hybrid samples	n	assignment at $q > 0.2$	<i>E. ovata</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
pedigreed F1	1	hybrid	0.5365	(0.266,0.804)	0.4635	(0.196,0.734)
pedigreed F1	1	hybrid	0.5316	(0.273,0.788)	0.4684	(0.212,0.727)

Table 5. Assignment of simulated, pedigreed and putative hybrid samples between *E. viminalis* and *E. globulus* obtained from STRUCTURE using a q cut-off of 0.2. Putative hybrid[?] = putative hybrid samples collected from mature trees in native forest where the generation is unknown.

Simulated samples	n	% correctly assigned at $q > 0.2$	<i>E. viminalis</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
Simulated <i>E. viminalis</i>	50	88	0.868	(0.886,0.849)	0.132	(0.151,0.114)
Simulated <i>E. globulus</i>	50	88	0.133	(0.151,0.115)	0.867	(0.885,0.849)
Simulated F1	50	100	0.507	(0.544,0.470)	0.493	(0.530,0.456)
Simulated F2	50	90	0.487	(0.537,0.438)	0.513	(0.562,0.462)
Simulated BCv	50	78	0.717	(0.749,0.688)	0.281	(0.312,0.251)
Simulated BCg	50	76	0.284	(0.314,0.254)	0.716	(0.746,0.685)
Total	300	86.7				
Pedigreed and putative hybrids	n	assignment at $q > 0.2$	<i>E. viminalis</i> cluster		<i>E. globulus</i> cluster	
			mean q	95% CI (+ -)	mean q	95% CI (+ -)
pedigreed F1	1	hybrid	0.6896	(0.378,0.944)	0.3104	(0.056,0.622)
pedigreed F1	1	hybrid	0.5601	(0.276,0.840)	0.4399	(0.160,0.724)
pedigreed F1	1	hybrid	0.4754	(0.200,0.759)	0.5246	(0.241,0.800)
putative hybrid [?]	1	hybrid	0.3296	(0.104,0.591)	0.6704	(0.409,0.896)
putative hybrid [?]	1	hybrid	0.2058	(0.019,0.464)	0.7942	(0.536,0.981)
putative hybrid [?]	1	hybrid	0.3522	(0.083,0.654)	0.6478	(0.346,0.917)
putative hybrid [?]	1	<i>E. globulus</i>	0.1137	(0.002,0.355)	0.8863	(0.645,0.998)