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Supplementary Materials for

Inheritance of somatic mutations by animal offspring

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Data S1 to S10

Supplementary Notes

Note 1: Chimeras

Coral chimeras harbor two or more diploid genomes. While they have been described in the *Acropora* genus (71, 72), they are rare in *A. palmata* (24). A previous study characterized only three chimeras (2.2%) out of 90 *A. palmata* genets with at least five ramets (*n*=1,294 samples in total) (24). Here, we assessed whether the mutations we observed were due to the parent genet being a chimera. To do so, we must consider the different ways in which chimeras in colonial invertebrates such as corals can form. In integrated chimeras, one of the genotypes may only be present in part of the cells whereas most cells contain the other genotype. For example, chimeric brooding corals can release uniparental, asexual larvae with mixed proportions of the parental genotypes (73). In other cases of coral chimeras, portions of the colony may be of one genotype while other portions of the colony may be of a different genotype because of fusion of juvenile recruits. Chimeras developing from broadcast spawners like *A. palmata* are more likely to form due to fusion events of closely related recruits (ie., full or half siblings). The resulting mature colony consists of neighboring groups of polyps from different genets (*60, 72*).

We ruled out the possibility that the parent genet was a chimera for three reasons:

First, a chimera between two siblings or unrelated genets should result in genetic distance values greater than between-ramet values (Fig. 1D and Data S2, (74)). The genetic distances among ramets of the parent genet were an order of magnitude lower $(0.0056 \pm 0.0030 \text{ (average} \pm \text{SD)})$ than the genetic distance among full siblings (0.0508 ± 0.0082) and among *A. palmata* genets (0.128 ± 0.025) (Fig. 1D, Data S2 and ref. 26). On average, the number of probes that differ between siblings is around 1,000, while the maximum number of probes that differed between

the sampled ramets from the parent colony was 52 (Table S1). Therefore, repeated sampling along the two main branches of the parent colony did not reveal evidence of two distinct genets as would be expected from a fusion event of siblings or unrelated recruits.

Second, we reasoned if the parent colony was a fully integrated chimera of siblings at the tissue level, then we would expect differences in the ploidy of the genomic DNA used for the SNP analysis. In humans, chimeras with differing amounts of two genomes have been detected using deviations in the total and relative genotyping array signal (*75*). For our sampled ramets, we did not detect evidence of genome-wide ploidy differences based on the array signal intensity, suggesting they are diploid (see Note 3 below and Data S7).

Third, if the observed mutations were the result of chimerism where the non-mutant somatic tissue is of one genotype and the mutant germline tissue is of another genotype, we would expect all germline variants to be inherited. Instead, only a subset (134 out of 268) were inherited.

Note 2: Detection limits of DNA mixtures

We experimentally tested the ability of the SNP microarray platform to detect when two genotypes are present in one sample, either through contamination or chimerism. Mixtures of extracted DNA from two siblings (pairwise genetic distance = 0.0709) were created to represent dominance of one genet (ratios of 85:15, M1) and nearly equal contribution of both genets (ratio of 52:42, M2). The mean nucleotide signals of C or G were significantly higher in the DNA mixtures relative to either Genet A, Genet B, the parental and neighbor ramets or the offspring (1-way ANOVA Tukey *post-hoc*, p < 0.05; Fig S3).

Other microarray metrics (heterozygosity, missing data, genetic distance, ploidy differences) were able to detect DNA mixtures when each donor contributed about equal to the mixture (M2) but were not able to detect a minor contribution of a second donor (M1).

Note 3: Mosaicism and Copy Number Variation

Given that each sample represented a non-chimeric mixture of different tissue (e.g., epidermis, gastrodermis) and cell types (e.g., sensory cells, cnidocytes), we sought to determine if the putative SMs were a product of deviations in array signal due to underlying cell mosaicism. Alternatively, array signal deviations could be caused by copy number variations (CNVs) between *A. palmata* and *A. digitifera*, the species used as the reference genome for the analysis, or between the parent genet and offspring samples.

We predicted CNVs using the median normalized log R allele signal intensity estimated for each SNP locus with the R package CGHcall (*66*) and through visual inspection. The log R ratio (LRR) is the relative signal intensity observed for each SNP locus over the expected signal intensity of two genomic copies. Deviations from zero will reflect genomic copy gains (LRR > 0) or losses (LRR < 0). For the *in silico* predictions, we found significantly more CNVs in the offspring (22.1% of all probes) than parent samples (9.3%; $\chi^2(1) = 1213.5$, p < 0.001), but no significant difference in the proportion of CNVs that were predicted SMs or inherited SMs between offspring and parents ($\chi^2(2) = 0.9703$, p = 0.6156; Fig. S5A and B). The CNVs were categorized into double deletion (no allele copies), hemizygous deletion (1 allele copy), normal (2 allele copies), gain (3 allele copies) and amplification (> 3 allele copies). Only hemizygous deletions and gains were detected in the parent and offspring samples, which may represent some level of cell mosaicism in the samples (Data S6). A heat map of CNVs plotted along the longest 1

to shortest scaffolds of the reference genome *A. digitifera* (Fig. S5C) illustrates that some CNVs were recovered in all uniparental offspring (n= 50 gain CNVs and n=1 loss CNV).

To assess if the *in silico* CNV calls were influenced by the fragmented genome assembly, we 4 visually inspected the log R ratio plots of all predicted SMs (n=268, Data S7). Our criteria for visually calling CNVs of the mutant carrying sample were as follows: A) LRR exceeded ± 0.5 and the BAF ~ 0.5 (double deletion), B) LRR exceeded ± 0.5 and the BAF = 0.4 or 0.6 (single deletion), and C) LRR exceeded ± 0.5 and the BAF = 0, 0.33, 0.67, or 1 (duplication: AAA, AAB, ABB, BBB, respectively) (76, 77). There were 95 SMs detected as a CNV by at least one method and 14 shared by both methods (Fig. S5E). No difference in copy number was detected in the remaining 173 SMs (Fig. S5E), of which 82 were inherited SMs (example in Fig. S6, Data 1 S6). The CNVs detected were a fraction (39%) of the inherited SMs.

Note 4: RFLP validation of SMs 3

Ten SNP-containing regions were amplified by PCR, and the resulting PCR product was digested by a restriction enzyme (Fig. 1C and Data S8). Two loci were detected to have some copy number variation and eight were without copy number variation (see Note 3). Markers that produced both sharp PCR bands and clear results in restriction digests were further investigated (*n*=2 markers out of 10). Of these markers, GOH variant mutation locus AX-212313644 produced the clearest banding patterns on gels. Two offspring (SWSA-179, SWSA-181), share the heterozygous mutation with the parent 453, resulting in 3 bands (Fig. 1C) while parent 455 predicted to have the non-mutant homozygous state for this site did not cut, resulting in 1 band (Fig. 1C).

The copy-number variant, AX-212294854, was also confirmed through this method (Fig. S7). The mutant allele for this probe was heterozygous, so that the expected banding pattern was three bands. The major allele for this probe was homozygous for the allele not recognized by the restriction enzyme, and therefore was expected to not be cut. There was significant observed STAR activity in this probe (more fragments than expected by the individual cut site). This could be explained by the observed copy number variation between the offspring and parent that share the heterozygous mutation (Data S6), resulting in the excess bands seen in Fig. S7. The RFLP marker confirms the inheritance of the GOH mutation found at this locus discovered in the SNP array data.

Not all identified mutations with designed assays were validated with this method. For the mutation of probe AX-197939655, the major allele shared by most samples was homozygous for the allele not recognized by the enzyme, and the mutant was heterozygous at that restriction site. The restriction enzyme did not cut the PCR product of the mutant offspring for this restriction site, suggesting that the mutant site was homozygous contrary to expectations.

Note 5: Somatic mutation detected in Florida larvae

The first indication that somatic mutations could be passed to offspring came from a 2017 observation where we detected known somatic mutations in microsatellite loci from offspring of two *A. palmata* genets from Florida (24). Most larvae (61.3%, n=39) were produced with genetic contributions from both parents, but 38.7% (n=24) contained genetic contributions from only one parent (uniparental larvae) (Data S10). It was unclear whether these offspring were the product of self-fertilization or parthenogenesis, but either way indicated that *A. palmata* colonies employ a wider range of reproductive modes than previously thought (*38, 54*). Somatic mutations were

detected in six uniparental larvae and one biparental larva (Fig. S7). The six uniparental larvae appeared to be diploid because they inherited two different alleles at one or more loci that were heterozygous in the parent.



Fig. S1. Summary of reproductive strategies of broadcast spawning corals. (A) *A. palmata* gametes are mostly self-incompatible so that fertilization occurs only between gametes from different genets (biparental outcross). However, self-fertilization and parthenogenic offspring were observed in this study. **(B)** Overview of the genetic consequences of the different reproductive modes on ploidy (n) and heterozygosity.





Fig. S2. SNP microarray performance on DNA mixtures of two genets. (A) Samples from *A. palmata* colonies are dominated by somatic tissue (orange). Germline tissue is restricted to mesenteries where gametes are produced (blue, inset) (78). The red star denotes the occurrence of a somatic mutation that has been fixed within a polyp. A DNA sample typically consists of 3-4 polyps mostly composed of somatic tissue. (B) Mixtures of DNA extracted from two siblings, Genet A and B, were created to represent dominance of one genet (ratio of 85:15, M1) and nearly equal contribution of both genets (ratio of 52:42, M2). (C) Mean signal intensities for each nucleotide (A, T, C and G) by group. Statistical differences from a Tukey *post hoc* test following a 1-way ANOVA are indicated by differing lowercase letters. The artificial DNA mixtures, M1 and M2 (M1: n=1, light purple and M2: n=1, dark purple) resulted in higher signal means than signal means from samples of the donor genets A and B (n=4, orange), the samples from the neighbor colonies (n=5, gray), the samples from the parent colony (n=10, black) or the uniparental offspring (n=30, blue).









Fig. S4. Deviations in array signal indicate potential copy number variation. (A) UpSet plot of loss (blue) or gain (orange) copy number variants (CNV) detected *in silico* for two groups, offspring (n=30) and parents (n=15). The horizontal bar plots show the total number of loci with *in silico* detected CNVs for each group. The asterisk indicates significance from a chi-square test, alpha level = 0.05. The vertical stacked bar plots show the number of shared CNVs for each group. Each bar is split by the proportion of non-mutant (grey), somatic mutations (SMs, dark blue) and inherited SMs (light blue). The connected colored circles below the plot show groups that share the number of CNVs indicated by the above bar. (**B**) The top right inset plot is the proportion of shared CNVs for each sample along the *A. digitifera* scaffolds. (**D**) Venn diagram of the SMs detected as CNVs (n=95) from *in silico* and visual inspection of the log R ratio plots (Data S8). The remaining SMs (n=173) were not predicted to be CNVs by either approach.





Fig. S5. An example of copy number variation of an inherited SM. B-allele frequency (top) and log R ratio (bottom) for the fifteen parent and thirty offspring samples for locus AX-212309463. The blue panel labels are those samples that share a predicted loss of heterozygosity SM and orange labels are those samples that share a gain of heterozygosity due to CNV.





Fig. S6. Inherited GOH mutation locus AX-212294854. (A) B-allele frequency and log R ratio of parent 3 and offspring 126 with a shared heterozygous mutation while other parent and offspring samples share the non-mutant homozygous state. The log R ratio of the parent and offspring sample that shared the mutation exceeded the 0.5 threshold for copy number changes, suggestive of a CNV at this locus. (B) RFLP validation of AX-212294854. For each sample, the uncut (U) and cut (C) PCR products are shown. Each gel contains a size standard (lane M, bp = base pairs). The heterozygous mutation resulted in the three predicted bands in the offspring 126 and parent 3, as well as several others presumably due to CNVs.

Fig. S7.



Fig.S7. Examples of inheritance of somatic mutations detected with microsatellites. Four distinct patterns of allelic inheritance were observed across five *Acropora*-specific microsatellite loci. Examples of these four patterns (L1, L2, L3 and L4) are depicted here. Gametes were collected from two hermaphroditic *A. palmata* colonies (P1 and P2) that each had known somatic mutations in one of the five loci assayed (166, 181, 182, 192, 207). Ancestral alleles are indicated by solid-colored blocks, mutated alleles are indicated by blocks with vertical black lines. While diploid in the ancestral state (represented as two blocks per locus), *A. palmata* ramets can gain alleles over time via gene duplication (three blocks per locus (*24*)). P1 and P2 were crossed. Allelic patterns in most resulting biparental larvae (larval cohort L1) followed Mendelian expectations (60.3%) while only one biparental larva, L2, was observed to have mutated allele. A subset of larvae (cohorts L3 and L4) was uniparental in origin. Larvae in cohorts L3 and L4 inherited alleles from only one parent (P2), including the somatic mutation at locus 166 in the L3 cohort. Note that L4 larvae could either be haploid or diploid.

Table S1. *A. palmata* **samples analyzed by the SNP array.** Group refers to the parental samples or offspring analyzed (Fig 1A). The affymetrix ID is the unique sample identification number and the coral multi-locus genotype identification (MLG ID) is the unique genet identification number from the STAGdb (26). The grey shaded MLG IDs were identified as the same genet. Percent missing data and heterozygosity were calculated from 19,696 genotyping SNPs. The number of SMs were tallied from the minority SNP allele calls of the parental genet and those shared by the offspring.

				%		#
			Coral	Missin	%	predicted
Sample ID	Group	Affy ID	MLG ID	g SNPs	heterozygosity	SMs
`	Parent colony;	a550962-4393310-	HG0432			
16065(B1)	Branch A	052921-062 A01.CEL		1.44	14.93	24
	Parent colony;	a550962-4393310-	HG0432			
16066(B2)	Branch A	052921-062 C01.CEL		1.08	14.67	14
	Parent colony:	a550962-4393310-	HG0432			
16067(B3)	Branch A	052921-062 E01.CEL		1.11	14.98	2
~ /	Parent colony;	a550962-4393310-	HG0432			
16068(B4)	Branch A	052921-062 G01.CEL		1.09	15.44	7
	Parent colony;	a550962-4393310-	HG0432			
16069(B5)	Branch A	052921-062 I01.CEL		1.12	15.97	18
	Parent colony;	a550962-4393310-	HG0432			
16070(B6)	Branch B	052921-062 K01.CEL		1.55	15.32	32
	Parent colony;	a550962-4393310-	HG0432			
16071(B7)	Branch B	052921-062 M01.CEL		1.22	15.68	20
	Parent colony;	a550962-4393310-	HG0432			
16072(B8)	Branch B	052921-062 O01.CEL		1.89	15.43	52
	Parent colony;	a550962-4393310-	HG0432			
16073(B9)	Branch B	052921-062 A03.CEL		1.13	15.27	8
	Parent colony;	a550962-4393310-	HG0432			
16074(B10)	Branch B	052921-062 C03.CEL		1.33	15.86	15
()	Neighboring	a550962-4393310-	HG0432			
16075(C1)	Colony 1	052921-062 E03.CEL		1.17	15.45	13
	Neighboring	a550962-4393310-	HG0432			
16076(C2)	Colony 2	052921-062 G03.CEL		1.28	15.33	22
	Neighboring	a550962-4393310-	HG0432			
16077(C3)	Colony 3	052921-062 I03.CEL		2.34	17.09	149
	Neighboring	a550962-4393310-	HG0432			
16078(C4)	Colony 4	052921-062 K03.CEL		0.98	14.56	18
	Neighboring	a550962-4393310-	HG0432			
16079(C5)	Colony 5	052921-062 M03.CEL		1.69	16.17	46
Offspring-	uniparental	a550962-4393310-	HG0421			
159	offspring	052921-062 M11.CEL		0.87	8.45	24
Offspring -	uniparental	a550962-4393310-	HG0422			
147	offspring	052921-062 O09.CEL		1.23	8.37	21
Offspring -	uniparental	a550962-4393310-	HG0424			
158	offspring	052921-062 K11.CEL		1.09	9.68	20
Offspring -	uniparental	a550962-4393310-	HG0427			
160	offspring	052921-062_011.CEL		1.23	9.38	22
Offspring -	uniparental	a550962-4393310-	HG0429			
145	offspring	052921-062_M09.CEL		0.85	8.68	<u>2</u> 6

Table S1 continued.

			a 1	0/) 5 · ·	0 /	#
G 1 ID	0		Coral	% Missing	%	predicted
Sample ID	Group	Affy ID	MLG ID	SNPs	heterozygosity	SMs
Offspring -	uniparental	a550962-4393310-	1100420	1.70	0.00	26
163	offspring	052921-062_A13.CEL	HG0430	1.72	9.80	26
Offspring -	uniparental	a550962-4393310-	1100422	2.24	10.07	1.4
144	offspring	052921-062_K09.CEL	HG0432	2.24	10.87	14
Offspring -	uniparental	a550962-4393310-	1100422	1.07	10.26	1.1
180	offspring	052921-062_M13.CEL	HG0432	1.97	10.36	11
Offspring -	uniparental	a550962-4393310-	1100422	1.40	0.12	10
	offspring	052921-062_M05.CEL	HG0433	1.43	9.12	18
Offspring -	uniparental	a550962-4393310-	1100424	1.24	7.05	26
16/	onspring	052921-062_E13.CEL	HG0434	1.24	7.05	26
Offspring -	uniparental	a550962-4393310-	1100425	0.92	0.05	20
142 Offensier	onspring	052921-062_109.CEL	HG0435	0.83	8.85	20
Olispring -	uniparentai	a550962-4595510-	1100426	1 24	0.09	20
102 Offensier	onspring	052921-062_003.CEL	HG0430	1.34	9.98	28
Olispring -	uniparentai	a550962-4595510-	1100427	1 15	0 57	16
11/ Offensier	onspring	052921-062_K05.CEL	HG0437	1.15	8.37	10
Ulispring -	uniparental	a550902-4595510-	1100429	1.22	0 62	12
119 Offenning	onspring	052921-062_005.CEL	HG0438	1.23	8.03	15
Unspring -	umparentai	a550902-4595510-	1100420	0.01	0.00	21
152 Offerning	onspring unimamental	052921-002_E11.CEL	ПО0439	0.91	9.00	21
Unspring -	offerring	052021 062 C12 CEL	UC0441	1.02	7 44	10
104 Offenning	uning	o550062 4202210	H00441	1.05	/.44	19
Offspring -	offerring	052021 062 012 CEI	UC0442	1 16	0.42	10
Offenring -	uniparental	2550062_/303310_	1100442	1.10	9.42	19
168	offenring	052921-062 G13 CEI	HG0443	2 17	0.80	18
Offenring -	uniparental	2550062_4303310_	1100445	2.17	9.00	10
127	offspring	052921-062 I07 CEI	HG0444	2.07	11.15	26
Offspring -	uniparental	a550962_4393310_	1100444	2.07	11.15	20
150	offspring	052921-062 C11 CEL	HG0448	1 27	9.05	22
Offspring -	uninarental	a550962-4393310-	1100440	1.27	2.05	
115	offspring	052921-062 I05 CFI	HG0449	1.68	8 84	32
Offspring -	uninarental	a550962-4393310-	1100447	1.00	0.01	52
157	offspring	052921-062 111 CEL	HG0453	1 22	912	26
Offspring -	uniparental	a550962-4393310-	1100100	1.22	<i></i>	20
126	offspring	052921-062 G07 CEL	HG0454	2.87	8 57	29
Offspring -	uniparental	a550962-4393310-	1100101	2.07	0.07	2)
138	offspring	052921-062 C09.CEL	HG0457	1.45	9.46	50
Offspring -	uniparental	a550962-4393310-	1100.07	1110	,	00
174	offspring	052921-062 I13.CEL	HG0460	1.28	9.79	33
Offspring -	uniparental	a550962-4393310-				
154	offspring	052921-062 G11.CEL	HG0461	1.10	10.08	28
Offspring -	uniparental	a550962-4393310-				
113	offspring	052921-062 E05.CEL	HG0462	1.14	9.18	21
Offspring -	uniparental	a550962-4393310-				
122	offspring	052921-062 A07.CEL	HG0463	0.97	9.39	14
Offspring -	uniparental	a550962-4393310-				
149 E2	offspring	052921-062 A11.CEL	HG0464	1.08	8.44	20
Offspring -	uniparental	a550962-4393310-				
179	offspring	052921-062_K13.CEL	HG0465	1.17	9.59	23

			~ 1	0/251	Â	#
	C		Coral	% Missing	% 1	predicted
Sample ID	Group	AITY ID	MLG ID	SNPS	heterozygosity	SIVIS
Olispring -	CUXFL	a550962-4595510-	1100422	0.02	15.09	
139 Offenning	CUTEI	052921-002_E09.CEL	ПО0423	0.92	15.08	n.a.
Olispring -	offenring	052021 062 M07 CEI	HG0428	1.07	16.06	n 0
Offenring -	CUvFI	2550062_/303310_	1100428	1.07	10.00	11.a.
135	offspring	052921_062_007_CEI	HG0445	1 35	16.12	na
Offspring -	CUxFI	a550962-4393310-	1100445	1.55	10.12	11.a.
124	offspring	052921-062 E07 CEL	HG0451	0.96	16.24	na
Offspring -	CUxFL	a550962-4393310-	1100451	0.90	10.24	m.a.
123	offspring	052921-062 C07.CEL	HG0425	1.19	16.26	n.a.
Offspring -	CUxFL	a550962-4393310-	1100125		10.20	mai
128	offspring	052921-062 K07.CEL	HG0446	0.96	16.96	n.a.
Offspring -	CUxFL	a550962-4393310-				
136	offspring	052921-062 A09.CEL	HG0447	1.3	16.97	n.a.
Offspring -	CUxFL	a550962-4393310-				
140	offspring	052921-062 G09.CEL	HG0450	1.06	17.00	n.a.
Offspring -	CUxFL	a550962-4393310-				
114	offspring	052921-062 G05.CEL	HG0455	1.37	17.12	n.a.
Offspring -	CUxFL	a550962-4393310-				
105	offspring	052921-062 A05.CEL	HG0452	1.49	17.24	n.a.
Offspring -	CUxFL	a550962-4393310-				
109	offspring	052921-062 C05.CEL	HG0456	1.8	17.34	n.a.
	1 0	a550962-43 6 8120-				
13931	Curacao	060520-252 I11.CEL	HG0194	0.59	12.82	n.a.
		a550962-43 6 8120-				
13927	Curacao	060520-252 I07.CEL	HG0193	1.53	13.45	n.a.
		a550962-4368120-				
13921	Curacao	060520-252_I03.CEL	HG0173	0.68	13.58	n.a.
		a550962-4368120-				
13937	Curacao	060520-252_I15.CEL	HG0172	1.21	13.86	n.a.
		a550962-4368120-				
13909	Curacao	060520-252_I01.CEL	HG0191	1.24	13.94	n.a.
		a550962-4368120-				
13929	Curacao	060520-252_I09.CEL	HG0176	0.76	14.25	n.a.
		a100000-4368120-				
13911	Curacao	060520-256_C17.CEL	HG0105	0.13	14.68	n.a.
100-00	~	a550962-4368120-		0.01		
13972	Curacao	060520-252_117.CEL	HG0168	0.81	14.77	n.a.
12010	C	a100000-4368120-	1100007	0.11	15 (0	
13919	Curacao	060520-256_117.CEL	HG0087	0.11	15.60	n.a.
12007	C	a100000-4368120-	1100100	0.07	15 (0	
13907	Curacao	060520-256_A17.CEL	HG0128	0.07	15.62	n.a.
12020	C	a100000-4368120-	110000	0.10	16 50	
13939	Curacao	060520-256_017.CEL	HG0082	0.10	16.58	n.a.
12022	Cumana	a100000-4308120-	1100001	0.14	16 60	
13933	Curacao	-100000 42(8120	HG0081	0.14	10.00	n.a.
1027	Florido	a100000-4508120-	11C0104	0.11	17.29	
1037	riorida	210000_/268120	HU0104	0.11	17.28	n.a.
2655	Florida	060520-256 CO3 CEI	HG0082	0.13	17 25	no
2033	TIOTIGA	2550962_/268120_	1100003	0.15	17.23	11.d.
5735	Florida	060520-256 K11 CFI	HG0080	0.26	13 70	na
5155	1 101100	000520 250_KILCEL	1100000	0.20	13.79	11.d.

Table S1 continued.

Table S1 continued.

Sample ID	Group	Affy ID	Coral MLG ID	% Missing SNPs	% heterozygosity	# predicted SMs
	orowp	2550962-4368120-		51115	neverezygeeney	51115
12634	Florida	060520-256_K17.CEL	HG0130	0.14	13.88	n.a.
1108	Florida	060520-256_M01.CEL	HG0108	0.13	15.86	n.a.
5757	Florida	060520-256_M11.CEL	HG0123	0.23	13.92	n.a.
12641	Florida	060520-256_M17.CEL	HG0130	0.28	14.07	n.a.
14743_Mixed	Florida	060520-256_M21.CEL	HG0004	0.48	13.89	n.a.
5774	Florida	060520-256_011.CEL 2550962-4368120-	HG0123	0.15	13.72	n.a.
12741	Florida	060520-256_017.CEL	HG0123	0.84	14.81	n.a.
SI-1.1	Florida	052921-062_A15.CEL 2550962-4393310-	HG0197	0.76	15.46	n.a.
1151_NF	Florida	052921-062_A23.CEL a550962-4368120-	HG0171	1.42	15.18	n.a.
13811	Belize	060520-252_G19.CEL a550962-4368120-	HG0187	1.15	12.88	n.a.
13835	Belize	060520-252_G21.CEL a550962-4368120-	HG0190	0.69	13.73	n.a.
15610	Belize	060520-252_K21.CEL a550962-4368120-	HG0189	0.78	14.30	n.a.
15611	Belize	060520-252_K23.CEL a550962-4368120-	HG0196	1.33	14.57	n.a.
15612	Belize	060520-252_M01.CEL a550962-4368120-	HG0195	0.67	14.20	n.a.
15619	Belize	060520-252_M15.CEL a550962-4368120-	HG0188	1.10	14.48	n.a.
15620	Belize	060520-252_M17.CEL a550962-4368120-	HG0203	0.70	13.60	n.a.
15622	Belize	060520-252_M21.CEL a550962-4368120-	HG0200	0.71	14.26	n.a.
15720	Belize	060520-252_019.CEL a550962-4368120-	HG0167	0.72	14.90	n.a.
15629	Belize	060520-253_A03.CEL a550962-4368120-	HG0250	0.59	15.24	n.a.
15630	Belize	060520-253_E07.CEL a550962-4368120-	HG0222	0.50	14.48	n.a.
15627	Belize Genet A1-	060520-253_I01.CEL a550962-4381376-	HG0244	0.61	14.60	n.a.
13949	Curacao Genet A2-	121220-857_A21.CEL a550962-4381376-	n.a	0.51	13.09	n.a
13970	Curacao 85:15 mix M1-	121220-857_C21.CEL a550962-4381376-	n.a	0.59	13.03	n.a
16532	Curacao 58:42 mix M2-	121220-857_019.CEL a550962-4381376-	n.a	0.61	13.01	n.a
16533	Curacao Genet B1-	121220-857_E21.CEL a550962-4381376-	n.a	2.27	14.48	n.a
13948	Curacao	121220-857_K19.CEL	n.a	0.95	13.90	n.a

						#
			Coral	% Missing	%	predicted
Sample ID	Group	Affy ID	MLG ID	SNPs	heterozygosity	SMs
	Genet B2-	a550962-4381376-				
13969	Curacao	121220-857_M19.CEL	n.a	0.53	13.30	n.a

Number of Parental Samples	Number of Mutations	Number of Inherited Mutations
9	1	N/A
8	2	N/A
7	6	6
6	4	4
5	3	3
4	7	7
3	15	12
2	29	14
1	204	88

Table S2. Number of mutations shared by samples of the parental genet. SNPs shared by seven or fewer samples were considered to be somatic mutations.

Data S1. Absolute genetic distance matrix between parents and uniparental offspring. (Excel Workbook)

Data S2. Somatic mutation alleles identified in SNP array samples. (Excel Workbook)

Data S3. Count of transitions, transversions, gains and loss of heterozygosity. (Excel Workbook)

Data S4. Predicted variant effects for the putative somatic mutations. (Excel Workbook)

Data S5. Mutations unique to uniparental offspring. For each probe (column 1), the offspring genotype in 0 and 1s is presented. The last two columns provide the scaffold ID and position for the SNP probe. (Excel Workbook)

Data S6. Frequency of parent genet or offspring with predicted gain or loss CNVs for each locus. (Excel Workbook)

Data S7. BAF and LRR plots for mutation-containing A. digitifera scaffolds (PDF)

Data S8. Restriction fragment length polymorphism design for 10 probes with putative inherited mutations. (Excel Workbook)

Data S9. Putative somatic mutations of the Curacao parental genet in the biparental offspring. The alleles of the Curacao genet mutations (n=268) were tracked through the Curacao x Florida biparental offspring (n=11) by comparing the observed versus expected proportion of alleles for the ancestral allele of both parent genets. Probes where observed allele frequencies match expected frequencies for the ancestral allele are colored white. Probes where the allele frequencies observed do not match expectations of the ancestral allele but could arise from the mutant allele are colored blue. Probes where allele frequencies observed do not match expectations of the ancestral allele solver observed do not match expectations of the ancestral allele frequencies (germline mutations, non-Mendelian inheritance, somatic mutation post-fertilization, or genotyping error) are colored orange. (Excel Workbook)

Data S10. Parental and offspring microsatellite allele sizes. Two copies of the same allele size are indicated by a slash (/). Reproduction status is inferred from the allele copies for each individual. The different observed alleles are denoted as al to an for each microsatellite marker, where n is the total number of unique alleles present. (Excel Workbook)

REFERENCES AND NOTES

- 1. A. Weismann, Das Keimplasma. Eine Theorie der Vererbung (Fisher, 1892).
- L. W. Buss, Evolution, development, and the units of selection. *Proc. Natl. Acad. Sci. U.S.A.* 80, 1387–1391 (1983).
- 3. M. Lynch, Evolution of the mutation rate. *Trends Genet.* 26, 345–352 (2010).
- L. Zhang, J. Vijg, Somatic mutagenesis in mammals and its implications for human disease and aging. *Annu. Rev. Genet.* 52, 397–419 (2018).
- 5. M. Kimura, On the evolutionary adjustment of spontaneous mutation rates. *Genet. Res.* **9**, 23–34 (1967).
- 6. L. W. Buss, The Evolution of Individuality (Princeton Univ. Press, 2014).
- 7. A. P. Bline, A. Le Goff, P. Allard, What is lost in the Weismann barrier? J. Dev. Biol. 8, 35 (2020).
- 8. E. Mayr, Weismann and evolution. J. Hist. Biol. 18, 295–329 (1985).
- 9. N. J. Berrill, C. K. Liu, Germplasm, Weismann, and Hydrozoa. Q. Rev. Biol. 23, 124–132 (1948).
- 10. L. W. Buss, Diversification and germ-line determination. *Paleobiology* 14, 313–321 (1988).
- 11. C. G. Extavour, M. Akam, Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. *Development* **130**, 5869–5884 (2003).
- 12. C. A. Whittle, C. G. Extavour, Causes and evolutionary consequences of primordial germ-cell specification mode in metazoans. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 5784–5791 (2017).
- 13. C. L. Littlefield, Germ cells in *Hydra oligactis* males. I. Isolation of a subpopulation of interstitial cells that is developmentally restricted to sperm production. *Dev. Biol.* **112**, 185–193 (1985).
- D. A. Gold, D. K. Jacobs, Stem cell dynamics in Cnidaria: Are there unifying principles? *Dev. Genes Evol.* 223, 53–66 (2013).

- C. Nishimiya-Fujisawa, T. Sugiyama, Genetic analysis of developmental mechanisms in *Hydra*: XX. Cloning of interstitial stem cells restricted to the sperm differentiation pathway in *Hydra magnipapillata*. *Dev. Biol.* **157**, 1–9 (1993).
- 16. I. E. Borisenko, M. Adamska, D. B. Tokina, A. V. Ereskovsky, Transdifferentiation is a driving force of regeneration in *Halisarca dujardini* (Demospongiae, Porifera). *PeerJ* **3**, e1211 (2015).
- 17. D. E. Wagner, I. E. Wang, P. W. Reddien, Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science* **332**, 811–816 (2011).
- 18. C. Juliano, G. Wessel, Versatile Germline Genes. Science 329, 640-641 (2010).
- E. H. López-Nandam, R. Albright, E. A. Hanson, E. A. Sheets, S. R. Palumbi, Mutations in coral soma and sperm imply lifelong stem cell renewal and cell lineage selection. bioRxiv 2021.07.20.453148 [Preprint]. 4 February 2022. https://doi.org/10.1101/2021.07.20.453148.
- 20. M. Schweinsberg, R. A. G. Pech, R. Tollrian, K. P. Lampert, Transfer of intracolonial genetic variability through gametes in *Acropora hyacinthus* corals. *Coral Reefs* **33**, 77–87 (2014).
- T. B. Reusch, I. B. Baums, B. Werner, Evolution via somatic genetic variation in modular species. *Trends Ecol. Evol.* 36, 1083–1092 (2021).
- 22. M. J. H. Van Oppen, P. Souter, E. J. Howells, A. Heyward, R. Berkelmans, Novel genetic diversity through somatic mutations: Fuel for adaptation of reef corals? *Diversity* **3**, 405–423 (2011).
- S. Goffredo, H. R. Lasker, Modular growth of a gorgonian coral can generate predictable patterns of colony growth. *J. Exp. Mar. Biol. Ecol.* 336, 221–229 (2006).
- M. K. Devlin-Durante, M. W. Miller; Caribbean *Acropora* Research Group, W. F. Precht, I. B. Baums, How old are you? Genet age estimates in a clonal animal. *Mol. Ecol.* 25, 5628–5646 (2016).
- E. H. Lopez, S. R. Palumbi, Somatic mutations and genome stability maintenance in clonal coral colonies. *Mol. Biol. Evol.* 37, 828–838 (2020).

- 26. S. A. Kitchen, G. Von Kuster, K. L. V. Kuntz, H. G. Reich, W. Miller, S. Griffin, N. D. Fogarty, I. B. Baums, STAGdb: A 30K SNP genotyping array and Science Gateway for *Acropora* corals and their dinoflagellate symbionts. *Sci. Rep.* 10, 12488 (2020).
- 27. M. Hagedorn, C. A. Page, K. L. O'Neil, D. M. Flores, L. Tichy, T. Conn, V. F. Chamberland, C. Lager, N. Zuchowicz, K. Lohr, H. Blackburn, T. Vardi, J. Moore, T. Moore, I. B. Baums, M. J. A. Vermeij, K. L. Marhaver, Assisted gene flow using cryopreserved sperm in critically endangered coral. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2110559118 (2021).
- E. F. Attiyeh, S. J. Diskin, M. A. Attiyeh, Y. P. Mossé, C. Hou, E. M. Jackson, C. Kim, J. Glessner, H. Hakonarson, J. A. Biegel, Genomic copy number determination in cancer cells from single nucleotide polymorphism microarrays based on quantitative genotyping corrected for aneuploidy. *Genome Res.* 19, 276–283 (2009).
- 29. I. W. Saunders, J. Brohede, G. N. Hannan, Estimating genotyping error rates from Mendelian errors in SNP array genotypes and their impact on inference. *Genomics* **90**, 291–296 (2007).
- 30. H. Hong, L. Xu, J. Liu, W. D. Jones, Z. Su, B. Ning, R. Perkins, W. Ge, K. Miclaus, L. Zhang, K. Park, B. Green, T. Han, H. Fang, C. G. Lambert, S. C. Vega, S. M. Lin, N. Jafari, W. Czika, R. D. Wolfinger, F. Goodsaid, W. Tong, L. Shi, Technical reproducibility of genotyping SNP arrays used in genome-wide association studies. *PLOS ONE* 7, e44483 (2012).
- 31. Y. G. Lee, N. Jeong, J. H. Kim, K. Lee, K. H. Kim, A. Pirani, B. K. Ha, S. T. Kang, B. S. Park, J. K. Moon, N. Kim, S.-C. Jeong, Development, validation and genetic analysis of a large soybean SNP genotyping array. *Plant J.* 81, 625–636 (2015).
- J. Engelstädter, Asexual but not clonal: evolutionary processes in automictic populations. *Genetics* 206, 993–1009 (2017).
- B. Charlesworth, Directional selection and the evolution of sex and recombination. *Genet. Res.* 61, 205–224 (1993).
- 34. H. J. Muller, Some genetic aspects of sex. Am. Nat. 66, 118–138 (1932).

- 35. S. Barfield, G. V. Aglyamova, M. V. Matz, Evolutionary origins of germline segregation in Metazoa: evidence for a germ stem cell lineage in the coral *Orbicella faveolata* (Cnidaria, Anthozoa). *Proc. R. Soc. B* 283, 20152128 (2016).
- 36. M. E. Orive, Somatic mutations in organisms with complex life histories. *Theor. Popul. Biol.* **59**, 235–249 (2001).
- I. B. Baums, M. W. Miller, M. E. Hellberg, Geographic variation in clonal structure in a reef building Caribbean coral, *Acropora palmata*. *Ecol. Monogr.* 76, 503–519 (2006).
- I. B. Baums, C. R. Hughes, M. H. Hellberg, Mendelian microsatellite loci for the Caribbean coral Acropora palmata. Mar. Ecol. Prog. Ser. 288, 115–127 (2005).
- G. M. Wessel, S. Morita, N. Oulhen, Somatic cell conversion to a germ cell lineage: A violation or a revelation? J. Exp. Zool. B Mol. Dev. Evol. 336, 666–679 (2021).
- 40. R. P. M. Bak, Y. Steward-Van Es, Regeneration of superficial damage in the scleractinian corals *Agaricia agaricites F. purpurea* and *Porites asteroides*. *Bull. Mar. Sci.* **30**, 883–887 (1980).
- P. M. Burton, J. R. Finnerty, Conserved and novel gene expression between regeneration and asexual fission in *Nematostella vectensis*. *Dev. Genes Evol.* 219, 79–87 (2009).
- 42. A. Sebé-Pedrós, B. Saudemont, E. Chomsky, F. Plessier, M.-P. Mailhé, J. Renno, Y. Loe-Mie, A. Lifshitz, Z. Mukamel, S. Schmutz, S. Novault, P. R. H. Steinmetz, F. Spitz, A. Tanay, H. Marlow, Cnidarian cell type diversity and regulation revealed by whole-organism single-cell RNA-Seq. *Cell* 173, 1520–1534.e20 (2018).
- 43. K. Kawamura, K. Nishitsuji, E. Shoguchi, S. Fujiwara, N. Satoh, Establishing sustainable cell lines of a coral, *Acropora tenuis*. *Marine Biotechnol.* **23**, 1–16 (2021).
- 44. M. Pineda-Krch, T. Fagerstrom, On the potential for evolutionary change in meristematic cell lineages through intraorganismal selection. *J. Evol. Biol.* **12**, 681–688 (1999).
- 45. J. T. Buchholz, Developmental selection in vascular plants. Bot. Gaz. 73, 249–286 (1922).

- 46. L. L. Whyte, Internal factors in evolution. Acta Biotheor. 17, 33–48 (1964).
- 47. A. Weismann, *On Germinal Selection as a Source of Definite Variation* (Open Court Publishing Company, 1896).
- 48. W. Roux, Der Kampf der Theile im Organismus (W. Engelmann, 1881).
- 49. N. Shakiba, A. Fahmy, G. Jayakumaran, S. McGibbon, L. David, D. Trcka, J. Elbaz, M. C. Puri, A. Nagy, D. van der Kooy, S. Goyal, J. L. Wrana, P. W. Zandstra, Cell competition during reprogramming gives rise to dominant clones. *Science* **364**, eaan0925 (2019).
- 50. Z. Bódi, Z. Farkas, D. Nevozhay, D. Kalapis, V. Lázár, B. Csörgő, Á. Nyerges, B. Szamecz, G. Fekete, B. Papp, H. Araújo, J. L. Oliveira, G. Moura, M. A. S. Santos, T. Székely Jr, G. Balázsi, C. Pál, Phenotypic heterogeneity promotes adaptive evolution. *PLOS Biol.* 15, e2000644 (2017).
- 51. S. Venkataram, B. Dunn, Y. Li, A. Agarwala, J. Chang, E. R. Ebel, K. Geiler-Samerotte, L. Hérissant, J. R. Blundell, S. F. Levy, Development of a comprehensive genotype-to-fitness map of adaptation-driving mutations in yeast. *Cell* 166, 1585–1596.e22 (2016).
- 52. J. R. Blundell, K. Schwartz, D. Francois, D. S. Fisher, G. Sherlock, S. F. Levy, The dynamics of adaptive genetic diversity during the early stages of clonal evolution. *Nat. Ecol. Evol.* **3**, 293–301 (2019).
- 53. N. D. Fogarty, S. V. Vollmer, D. R. Levitan, Weak prezygotic isolating mechanisms in threatened Caribbean *Acropora* corals. *PLOS ONE* **7**, e30486 (2012).
- 54. I. B. Baums, M. K. Devlin-Durante, N. R. Polato, D. Xu, S. Giri, N. S. Altman, D. Ruiz, J. E. Parkinson, J. N. Boulay, Genotypic variation influences reproductive success and thermal stress tolerance in the reef building coral, *Acropora palmata*. *Coral Reefs* **32**, 703–717 (2013).
- 55. C. G. Eckert, The loss of sex in clonal plants. Evol. Ecol. 15, 501–520 (2002).
- S. C. H. Barrett, Influences of clonality on plant sexual reproduction. *Proc. Natl. Acad. Sci. U.S.A.* 112, 8859–8866 (2015).

- 57. K. Bobiwash, S. T. Schultz, D. J. Schoen, Somatic deleterious mutation rate in a woody plant: estimation from phenotypic data. *Heredity* **111**, 338–344 (2013).
- D. E. Gill, L. Chao, S. L. Perkins, J. B. Wolf, Genetic mosaicism in plants and clonal animals. *Annu. Rev. Ecol. Syst.* 26, 423–444 (1995).
- 59. L. Yu, C. Boström, S. Franzenburg, T. Bayer, T. Dagan, T. B. H. Reusch, Somatic genetic drift and multilevel selection in a clonal seagrass. *Nat. Ecol. Evol.* **4**, 952–962 (2020).
- 60. E. Puill-Stephan, M. J. H. van Oppen, K. Pichavant-Rafini, B. L. Willis, High potential for formation and persistence of chimeras following aggregated larval settlement in the broadcast spawning coral, *Acropora millepora. Proc. R. Soc. B* 279, 699–708 (2012).
- 61. B. J. Knaus, N. J. Grunwald, VCFR: A package to manipulate and visualize variant call format data in R. *Mol. Ecol. Resour.* **17**, 44–53 (2017).
- 62. Z. N. Kamvar, J. F. Tabima, N. J. Grünwald, Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2**, e281 (2014).
- 63. A. Prevosti, J. Ocana, G. Alonso, Distances between populations of *Drosophila subobscura*, based on chromosome arrangement frequencies. *Theor. Appl. Genet.* **45**, 231–241 (1975).
- 64. M. H. Ferdosi, B. P. Kinghorn, J. H. J. van der Werf, S. H. Lee, C. Gondro, *hsphase*: An R package for pedigree reconstruction, detection of recombination events, phasing and imputation of half-sib family groups. *BMC Bioinformatics* **15**, 172 (2014).
- 65. Z. G. Gu, R. Eils, M. Schlesner, Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).
- 66. M. A. van de Wiel, K. I. Kim, S. J. Vosse, W. N. van Wieringen, S. M. Wilting, B. Ylstra, CGHcall: Calling aberrations for array CGH tumor profiles. *Bioinformatics* **23**, 892–894 (2007).
- 67. P. Cingolani, A. Platts, L. L. Wang, M. Coon, T. Nguyen, L. Wang, S. J. Land, X. Y. Lu, D. M. Ruden, A program for annotating and predicting the effects of single nucleotide polymorphisms,

SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w(1118); iso-2; iso-3. *Fly* **6**, 80–92 (2012).

- 68. C. Shinzato, E. Shoguchi, T. Kawashima, M. Hamada, K. Hisata, M. Tanaka, M. Fujie, M. Fujiwara, R. Koyanagi, T. Ikuta, A. Fujiyama, D. J. Miller, N. Satoh, Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* **476**, 320–323 (2011).
- 69. A. Untergasser, I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, S. G. Rozen, Primer3—New capabilities and interfaces. *Nucleic Acids Res.* **40**, e115 (2012).
- A. R. Quinlan, I. M. Hall, BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842 (2010).
- D. W. dela Cruz, P. L. Harrison, Enhanced larval supply and recruitment can replenish reef corals on degraded reefs. *Sci. Rep.* 7, 13985 (2017).
- 72. E. Puill-Stephan, B. L. Willis, L. van Herwerden, M. J. H. van Oppen, Chimerism in wild adult populations of the broadcast spawning coral *Acropora millepora* on the Great Barrier Reef. *PLOS ONE* 4, e7751 (2009).
- 73. B. Rinkevich, L. Shaish, J. Douek, R. Ben-Shlomo, Venturing in coral larval chimerism: A compact functional domain with fostered genotypic diversity. *Sci. Rep.* **6**, 19493 (2016).
- 74. K. O. Amar, N. E. Chadwick, B. Rinkevich, Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evol. Biol.* **8**, 126 (2008).
- 75. R. Bottega, S. Cappellani, A. Fabretto, A. M. Spinelli, G. M. Severini, M. Aloisio, M. Faleschini, E. Athanasakis, I. Bruno, F. Faletra, V. Pecile, Could a chimeric condition be responsible for unexpected genetic syndromes? The role of the single nucleotide polymorphism-array analysis. *Mol. Genet. Genomics* 7, e546 (2019).
- 76. R. Salomon-Torres, V. M. Gonzalez-Vizcarra, G. E. Medina-Basulto, M. F. Montano-Gomez, P. Mahadevan, V. H. Yaurima-Basaldua, C. Villa-Angulo, R. Villa-Angulo, Genome-wide identification

of copy number variations in Holstein cattle from Baja California, Mexico, using high-density SNP genotyping arrays. *Genet. Mol. Res.* **14**, 11848–11859 (2015).

- 77. D. A. Peiffer, J. M. Le, F. J. Steemers, W. H. Chang, T. Jenniges, F. Garcia, K. Haden, J. Z. Li, C. A. Shaw, J. Belmont, S. W. Cheung, R. M. Shen, D. L. Barker, K. L. Gunderson, High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. *Genome Res.* 16, 1136–1148 (2006).
- C. C. Wallace, Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. *Mar. Biol.* 88, 217–233 (1985).