Mutations in coral soma and sperm imply lifelong stem cell differentiation

Elora H. López-Nandam[1,2*], Rebecca Albright[2], Erik A. Hanson[1], Elizabeth A. Sheets[1,†], Stephen R. Palumbi[1]

[1]Biology Department, Hopkins Marine Station of Stanford University, Pacific Grove, California 93950 USA

[2] Institute for Biodiversity and Sustainability Science, California Academy of Sciences, San Francisco, California, 94118, USA

[†] Current address: Genomics Institute, University of California, Santa Cruz, 95064, USA

*corresponding author

In many animals, the germline differentiates early in embryogenesis, so only mutations that accumulate in germ cells are inherited by offspring[1]. Exceptions to this developmental process may indicate that other mechanisms have evolved to limit the effects of deleterious mutation accumulation[2]. Stony corals are animals that can live for hundreds of years[3] and have long been thought to produce gametes from somatic tissue[4]. To clarify conflicting evidence about germline-soma distinction in corals, we sequenced high coverage, full genomes with technical replicates for parent coral branches and their sperm pools. We identified single nucleotide variants (SNVs) unique to each parent branch, then checked if each SNV was shared by the respective sperm pool: 26% of post-embryonic SNVs were shared by the sperm and 74% were not. We also identified germline SNVs, those that present in the sperm but not in the parent. These data suggest that self-renewing stem cells in corals differentiate into germ and soma throughout the adult life of the colony, with SNV rates and patterns differing markedly in stem, soma, and germ lineages. In addition

to informing germline evolution, these insights inform how corals may generate adaptive diversity necessary in the face of global climate change.

In many animals, germ and somatic cells differentiate early in the embryonic stage, leading to separate lineages. Weismann first hypothesized this process in 1889 to explain why mutations that accumulate in somatic tissues during an animal's lifetime– including those that cause cancer- are not inherited by that animal's offspring. Instead, only mutations in germ cells, which undergo fewer cell divisions and have lower mutation rates, are inherited[5,6]. Since Weismann, embryonic germ-soma separation has been shown in vertebrates and many other animal taxa, but not in plants or in some animal groups, including cnidarians, sponges, tunicates, and platyheminths[7,8].

Animal exceptions to Weismann's Germ Plasm Theory are intriguing because they may have novel mechanisms to reduce the number of deleterious mutations inherited by sexually produced offspring. Moreover, such exceptions may signal the potential existence of stem cell lineage types not seen in vertebrates. The model cnidarians *Hydra* and *Hydractinia* possess interstitial stem cells, denoted i-cells, that can differentiate into both germ and soma during adult life[9,10]. A few models have hypothesized how heritable post-embryonic mutations may affect the gamete pool[11–13], but there is very little data on the pattern of somatic mutations and their inheritance in long-lived animals[14].

Clonal, colonial corals can live for hundreds to thousands of years, and were long thought to generate gametes from the somatic cells of clonal polyps[4]. Coral colonies accumulate somatic mutations at a rate similar to noncancerous human tissues[14]. If these mutations are inherited by the coral's gametes, they must increase the heritable mutational load of these animals. Some previous studies identified putative somatic mutations in the gametes or juvenile offspring of mutant parents[15,16], but others have reported absence of somatic mutations in the gametes[17].

49 These studies tracked few mutations, ranging from 9 to 170, and none detected germline
50 mutations in gametes or offspring. Only one verified that their putative mutations were not PCR
51 or sequencing error[17]. Here, we interrogated full genomes of multiple branches from multiple
52 coral colonies and their sperm. We identified germline variants in the sperm as well as post-
53 embryonic variants in the parent. The data reject the hypothesis that somatic cells give rise to
54 germ cells in corals, and reject the hypothesis that corals possess embryonic germline
55 differentiation. We hypothesize that both parent tissue and sperm arise from a common stem cell
56 lineage that proliferates and differentiates throughout the long lives of these animals.

57 To clarify the inheritance of mutations and the presence of germline-soma distinction in
58 *Acropora hyacinthus*, we removed branches from soon-to-spawn adult coral colonies and placed
59 them into individual cups of seawater (Extended Data Fig. 1). Each branch released gamete
60 bundles into its respective cup 20 minutes later (Fig 1a, Extended Data Fig. 1b). We extracted
61 DNA from each branch and each sperm pool, then constructed two replicate full genome
62 libraries from each DNA extraction (Fig 1b). To be verified, a SNV had to be present in both
63 replicate libraries of a given sample. The technical replicates eliminated over 90% of putative
64 SNVs that would have been called if we had used one library per sample, although the exact
65 number varied by SNV category (Extended Data Figs. 2, 3).

66 We identified four different types of SNVs: those that were unique to the polyps from a
67 single parent branch in a colony but were not detected in the sperm from that branch (Parent
68 Only) (1c), those that were found in just a single parent branch in a colony and were also shared
69 by the sperm from that branch (Parent and Sperm) (1d), those that were unique to a single sperm
70 pool in a colony and not present in any branch of the colony (Single Sperm Pool) (1e), and those

71 that were shared by all sperm pools in a colony but had never been seen in the polyps from any
72 branch (All Sperm Pools) (1f).

73 We assayed nine parent polyp samples, and the respective sperm pools for seven of those
74 samples, across three different colonies. The average depth of coverage across the genome was
75 40.6 ±3.1 (1 s.e.m) for the parent polyp libraries and 65.2 ±6.9 (1 s.e.m) for the sperm pool
76 libraries (Supp. Table 1). Across the full dataset we identified 2,356 SNVs, and all but one were
77 at unique sites, indicating that the SNVs called were not a result of consistent mapping error or
78 bias (Supp. Table 2). Each SNV was classified as a Gain of Heterozygosity (GoH), in which the
79 aberrant sample was a new heterozygote and all others were homozygous, or a Loss of
80 Heterozygosity (LoH), in which the aberrant sample was homozygous and the other samples
81 were heterozygous.

82 We identified 146-351 post-embryonic SNVs per parent branch (Supp. Table 1), and we
83 found that on average 25.7% ±3.7% (1 s.e.m.) SNVs were shared between a parent branch and
84 its respective sperm pool (Parent and Sperm, labelled P+S in Fig. 2a), whereas 74.3% ±3.7% (1
85 s.e.m.) post-embryonic SNVs found in a branch were not in the sperm (Parent Only, labelled PO
86 in Fig. 2a). These findings contradict the hypothesis from the Germ Plasm Theory that post-
87 embryonic mutations would not be found in the sperm at all, as well as the common assumption
88 that all coral somatic cells can produce gametes.

89 We found 50-145 post-embryonic SNVs in every sperm pool (Supp. Table 1). Of these
90 39.2% ±3.5% were shared with its parent branch (Parent and Sperm), and 52.2% ±5.4% were
91 found only in the sperm pool (Single Sperm Pool Only) (Fig. 2b). A small number of sperm
92 SNVs, 8.5% ±2.1%, were found in all sperm pools from that colony but none of the parent
93 samples that spawned them (All Sperm Pools, labelled ASP, Fig. 2b). That 2 out of every 5

94 SNVs present in a given sperm pool are post-embryonic, non-germline variants indicates that the
95 lack of an embryonic germline increases the number of SNVs in a colony's gametes by 66%,
96 compared to what the diversity would have been if the germline were segregated at the
97 embryonic stage. This may help explain the high degree of heterozygosity in many stony coral
98 species, though it is not yet known what fraction of these SNVs are too deleterious to survive
99 into adulthood.

100 The rate of SNVs per bp was significantly higher across the full callable genome than the
101 rate of SNVs in the callable coding regions of the genome for all SNV types (Fig. 2c) (see Table
102 1 for all means and Wilcoxon signed-rank test results). This may indicate that there is stronger
103 purifying selection against SNVs in coding regions than in non-coding regions of the genome, or
104 it may be a result of higher mismatch repair in exons[18].

105 We examined the spectrum of mutations, the relative proportions of mutations in
106 different classes, and found no significant differences in spectra among parent only, shared, and
107 germ line specific mutations (Extended Data Fig. 5). These data confirm lack of a signature of
108 UV-associated mutations in corals[13], which is intriguing considering that these colonies grow in
109 high UV conditions, and in highly oxygenated warm water[19].

110 Losses of heterozygosity tend to arise as a result of gene conversion due to homologous
111 recombination, a form of double stranded DNA break repair[20]. Consistent with previous
112 findings[14], 38.3 % ±3.0 % (1 s.e.m.) of all parent SNVs being GoH and 61.7 ±3.0% (1 s.e.m.)
113 being LoH. SNVs that were shared by the parent branch tissues and the sperm had a much higher
114 fraction of GoH and lower fraction of LoH (73.8 ±3.6% and 26.2 ±3.6%, respectively) than did
115 parent SNVs that were not found in the sperm (25.2 ±2.4% and 74.8 ±2.4%, respectively)
116 (Wilcoxon signed-rank test, V=28, p = 0.015) (Fig. 2d). SNVs found in just a single sperm pool

117 had approximately equal proportions of each type, 51.1% ±2.5% GOH and 48.9 ±2.5% LOH
118 SNVs. High LOH in soma that is not inherited by the sperm could be due to high incidence of
119 double-strand breaks in somatic cells exposed to high light and photosynthetically derived
120 oxidation, or high LOH levels in the parent-only SNVs may reflect stronger selection against
121 GOH than LOH in differentiated somatic cells.

122 To explore the role of selection on patterns of genome change, we compared the rates of
123 missense and synonymous SNVs across four classes: all somatic SNVs, parent-only SNVs,
124 shared parent and sperm SNVs, and SNVs found in a single sperm pool only. There were no
125 coding SNVs in the all sperm pool category. The average rate of coding mutations was highest in
126 parent only SNVs ($6.0 \times 10^{-7} \pm 1.6 \times 10^{-7}$, Extended Data Fig. 6a). The percent of coding
127 mutations that were missense was higher in single sperm pool SNVs (73.7 ±11.8%) than in the
128 other categories (55.2 ±6.8% all somatic, 51.5 ±10.7% parent only, 47.6 ±14.6% parent and
129 sperm, Extended Data Fig. 6b). The higher mean percent missense in SSPO was not statistically
130 significant, likely attributable to the small number of coding mutations in each category. Like
131 most studies on somatic mutations to date, the small number of coding mutations in this study
132 (94) leaves us underpowered to detect selection[21]. However, the fairly consistent pattern of more
133 missense mutations in sperm pool samples than somatic samples provides a first hint that the
134 SNVs in the soma may experience stronger negative selection than germline SNVs.

135 If we had found only separate parent and sperm SNVs this would have shown that
136 *Acropora* corals have classical Weismannian germ and somatic cell lineage differentiation at the
137 embryonic stage, which has been suggested previously[17]. Likewise, if we had found that all
138 parent tissue SNVs were also in the sperm, we would have concluded that *Acropora* corals
139 developed gametes directly from those tissues[15,16]. However, 74% of the SNVs that we identified

140 in parent tissue were also present in the sperm spawned from that branch, and 26% were not.
141 Based on these data, we hypothesize that in colonial corals, shared parent and sperm SNVs
142 derive from mutations in a common ancestor stem cell lineage that self-renews and proliferates
143 through the colony, and that eventually differentiates into both germ and soma throughout the
144 colony's adult life. This type of lineage (i-cells) has been described in Hydrozoan cnidarians[9,10].
145 Although i-cells have not yet been identified in corals, cells that look like the i-cells are present
146 in larvae of the coral *Acropora millepora*[22]. Our data suggest that branch-specific SNVs shared
147 in germ and somatic cells first arose in an i-cell lineage proliferating in that branch, and then
148 differentiated into germ and soma (Fig. 3a). SNVs found only in the parent but not in the sperm
149 would have arisen in terminally-differentiated somatic cells that cannot produce gametes, and
150 SNVs found only in the sperm would have arisen from differentiated germ cells (Figure 3a).
151 We hypothesize that the program of sequential germ line differentiation during adult life
152 shown in Hydrozoans is likely a conserved trait across Cnidaria and was present in the cnidarian
153 common ancestor. Germ and soma differentiation appears to happen locally, resulting in evident
154 mosaicism in every branch (Fig. 2a, Fig. 3b). Solana[8] made a similar prediction for planarians–
155 when stem cells can differentiate into both soma and germ cells, mutations that appear in both
156 the soma and the germline are derived from mutations in those stem cells.
157 Weismann's original germ cell theory proposed a mechanism whereby mutations
158 accumulated during an individual's lifetime would not be inherited by its offspring. In the case of
159 corals, our data show that some post-embryonic SNVs that occur in different branches within a
160 colony are inherited by its sperm. If i-cell SNVs are subject to selection, then the selection
161 regime that growing i-cell lines face could select for novel beneficial changes as well as select
162 against deleterious ones [2,23]. Reef building corals are extremely sensitive to small increases in

163 temperature, but these environmental changes frequently result in the death of just part of a
164 colony. If partial survival of a colony is the result of selection for post-embryonic SNVs in
165 different parts of the colony, then adaptation to environmental change may occur over the
166 lifetime of a single colony. If some of those post-embryonic SNVs are inherited by the surviving
167 polyps' gametes, then this may be an alternative, rapid route to adaptation for corals.

168 Our data suggest that anthozoans have i-cells that self-renew and remain multipotent
169 throughout the adult lifespan, which has previously been described in medusozoans. We also
170 show, for the first time, the genome-level consequences of SNVs in i-cells on the mutation load
171 of a long-lived animal species that lacks an embryonic germline. SNVs in the stem cell lines of a
172 coral colony increase the number of SNVs in the sperm by 66%. This may help to explain the
173 high degree of heterozygosity and adaptive polymorphism in many stony coral species.
174 Mechanisms that corals use to avoid mutational meltdown in long-lived cell lineages might
175 include consistent screening by natural selection in proliferating cell lines, or yet-to-be
176 discovered controls on coding gene mutation rates.

177 **Acknowledgements**

178 EHLN was funded by NSF GRFP and a Stanford Graduate Fellowship in Science and
179 Engineering (Morgridge Family Foundation). Research funded by grants from the Chan-
180 Zuckerberg BioHub and NSF OCE-1736736. We thank N. Neff at CZ-BioHub for sequencing
181 power and A. Voskoboynik P. Bump, and B. Cornwell for stem cell insights. Thanks to R.
182 Ritson-Williams, R. Ross, and BIOTA for field collection support, and thanks to the staff of the
183 Steinhart Aquarium for taking care of the corals.

184 **References**

185 1. Saffman, E. E. & Lasko, P. Germline development in vertebrates and invertebrates. *Cell.*

186 *Mol. Life Sci. C. 1999 558* **55**, 1141–1163 (1999).

187 2. Buss, L. W. Evolution, development, and the units of selection. *Proc. Natl. Acad. Sci. U.*
188 *S. A.* **80**, 1387–91 (1983).

189 3. Bythell, J. C., Brown, B. E. & Kirkwood, T. B. L. Do reef corals age? *Biol. Rev* 0–0
190 (2017). doi:10.1111/brv.12391

191 4. van Oppen, M. J. H., Souter, P., Howells, E. J., Heyward, A. & Berkelmans, R. Novel
192 Genetic Diversity Through Somatic Mutations: Fuel for Adaptation of Reef Corals?
193 *Diversity* **3**, 405–423 (2011).

194 5. Lynch, M. Evolution of the mutation rate. *Trends Genet.* (2010).
195 doi:10.1016/j.tig.2010.05.003

196 6. Moore, L. *et al.* The mutational landscape of human somatic and germline cells 1 Author
197 List 2. *bioRxiv* 2020.11.25.398172 (2020). doi:10.1101/2020.11.25.398172

198 7. Whitham, T. G. & Slobodchikoff, C. N. Evolution by individuals, plant-herbivore
199 interactions, and mosaics of genetic variability: The adaptive significance of somatic
200 mutations in plants. *Oecologia* **49**, 287–292 (1981).

201 8. Solana, J. Closing the circle of germline and stem cells: The Primordial Stem Cell
202 hypothesis. *Evodevo* **4**, (2013).

203 9. DuBuc, T. Q. *et al.* Transcription factor AP2 controls cnidarian germ cell induction.
204 *Science (80-.).* **367**, 757–762 (2020).

205 10. Juliano, C. E., Swartz, S. Z. & Wessel, G. M. A conserved germline multipotency
206 program. *Development* **137**, 4113–4126 (2010).

207 11. Orive, M. E. Somatic Mutations in Organisms with Complex Life Histories. *Theor. Popul.*
208 *Biol.* **59**, 235–249 (2001).

209 12. Klekowski, E. J. & Godfrey, P. J. Ageing and mutation in plants. *Nature* **340**, 389–391
210 (1989).
211 13. Hanlon, V. C. T., Otto, S. P. & Aitken, S. N. Somatic mutations substantially increase the
212 per-generation mutation rate in the conifer *Picea sitchensis*. *Evol. Lett.* (2019).
213 doi:10.1002/evl3.121
214 14. López, E. H. & Palumbi, S. R. Somatic Mutations and Genome Stability Maintenance in
215 Clonal Coral Colonies. *Mol. Biol. Evol.* **37**, 828–838 (2020).
216 15. Schweinsberg, M., González Pech, R. A., Tollrian, R. & Lampert, K. P. Transfer of
217 intracolonial genetic variability through gametes in Acropora hyacinthus corals. *Coral*
218 *Reefs* **33**, 77–87 (2014).
219 16. Vasquez Kuntz, K. L. *et al.* Title: Juvenile corals inherit mutations acquired during the
220 parent's lifespan Running Title: Juvenile corals inherit mutations acquired during the
221 parent's 2 lifespan 3 4. *bioRxiv* 2020.10.19.345538 (2020).
222 doi:10.1101/2020.10.19.345538
223 17. Barfield, S., Aglyamova, G. V. & Matz, M. V. Evolutionary origins of germline
224 segregation in Metazoa: evidence for a germ stem cell lineage in the coral *Orbicella*
225 *faveolata* (Cnidaria, Anthozoa). *Proc. R. Soc. B Biol. Sci.* **283**, 20152128 (2016).
226 18. Frigola, J. *et al.* Reduced mutation rate in exons due to differential mismatch repair. *Nat.*
227 *Genet.* ng.3991 (2017). doi:10.1038/ng.3991
228 19. Shick, J. M., Lesser, M. P. & Jokiel, P. L. Effects of ultraviolet radiation on corals and
229 other coral reef organisms. *Glob. Chang. Biol.* **2**, 527–545 (1996).
230 20. Takata, M. *et al.* Homologous recombination and non-homologous end-joining pathways
231 of DNA double-strand break repair have overlapping roles in the maintenance of

chromosomal integrity in vertebrate cells. *EMBO J.* **17**, 5497–508 (1998).

21. Martincorena, I. *et al.* Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell* **171**, 1029-1041.e21 (2017).

22. Reyes-Bermudez, A. & Miller, A. D. J. In vitro culture of cells derived from larvae of the staghorn coral Acropora millepora. doi:10.1007/s00338-009-0527-3

23. Otto, S. P. & Orive, M. E. Evolutionary consequences of mutation and selection within an individual. *Genetics* **141**, (1995).

Figures and Legends

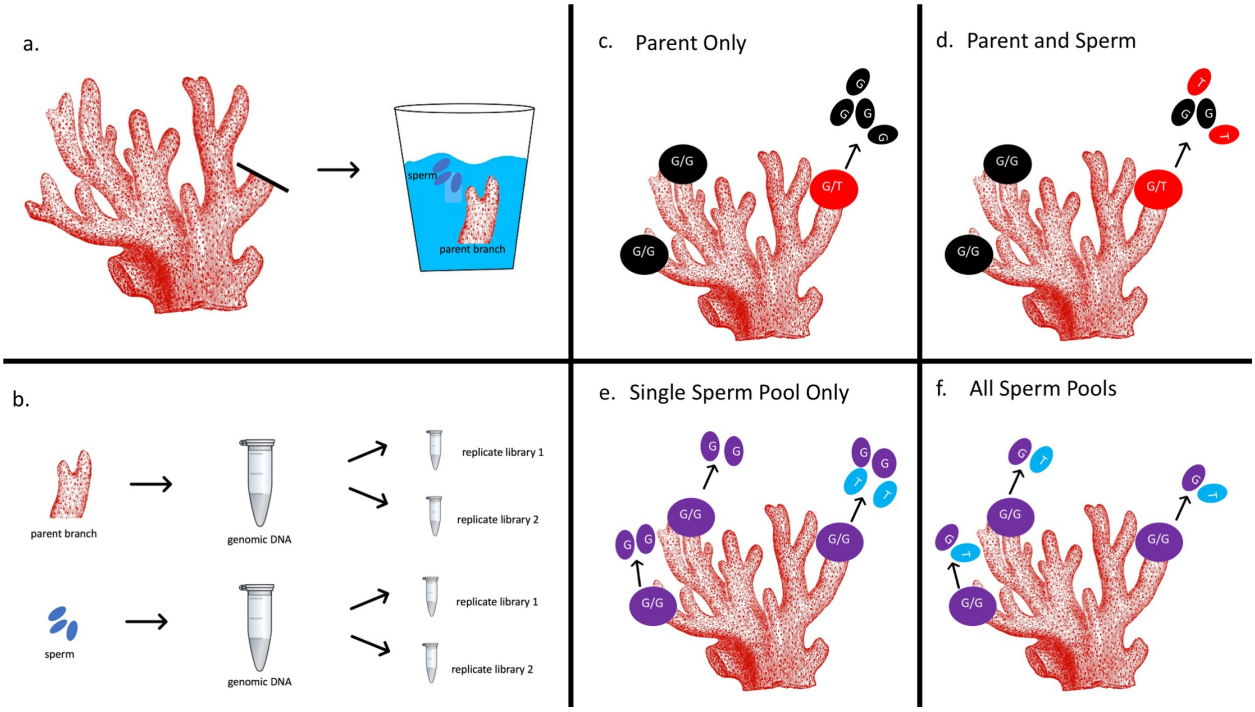


Figure 1. Data collection (a-b) and mutation classification (c-f). a.) Twenty minutes prior to spawning, 3-4 branches were broken off of 3 parent colonies and placed into individual cups of seawater, for a total of 10 branches in cups. Branches then released eggs and sperm into each cup, and sperm was collected from the cup. Both the sperm pool and the parent samples were stored in RNAlater and frozen. b.) Genomic DNA was extracted from each parent branch and sperm pool (see Methods). For each genomic DNA extraction we constructed two full genome libraries (see Methods) for technical replication. Classification of mutation types: c.) A mutation unique to a single branch of the colony, but the sperm from the branch does not share the mutant genotype. d.) A mutation unique to a single branch of the colony, and the sperm from the branch shares the mutant genotype. e.) A mutation unique to just one sperm pool in the colony, not

shared by other sperm pools or the parent branches. f.) A mutant genotype shared by all sperm pools from a particular colony, but none of the parent branches in that colony.

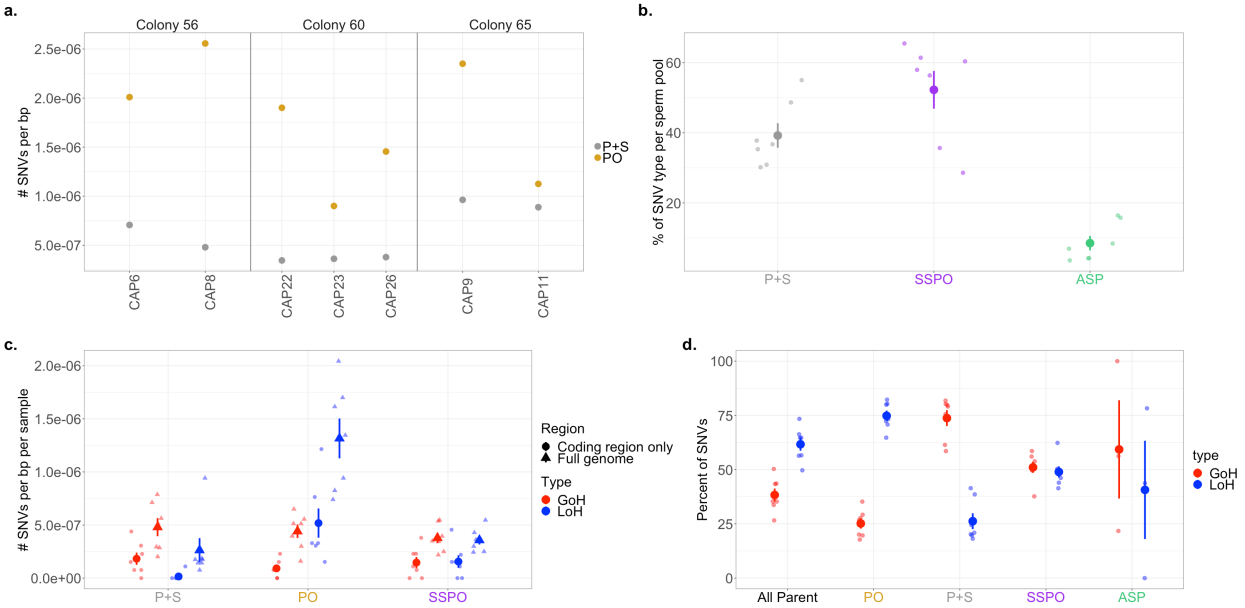


Figure 2. SNV rates and proportions across different classifications. a.) The rate of SNVs per bp for two SNV types: shared by parent and sperm (gray) and found in the parent only (yellow) for the seven parent-sperm pairs from the three colonies. b.) The average percentage of SNV type (parent and sperm, P+S), (parent only, PO), and all sperm pools (ASP) found in each sperm pool sample (N=7). c.) The average rate of SNVs per bp per sample (N=7) across the full genome and for the coding regions only, for three SNV types: parent and sperm (P+S), parent only (PO), and single sperm pool only (SSPO). For each SNV type, each subtype (GoH and LoH). d.) The average percentage of SNVs that were GoH and LoH for each of the four SNV types found in each sample (N=7). For b, c, and d, the mean for each category is shown as a large point with error bars extending out; error bars represent ±1 s.e.m. Each individual data point (N=7 for P+S, SSPO, PO, and All Parent, N=3 for ASP) is shown as a smaller point for each category.

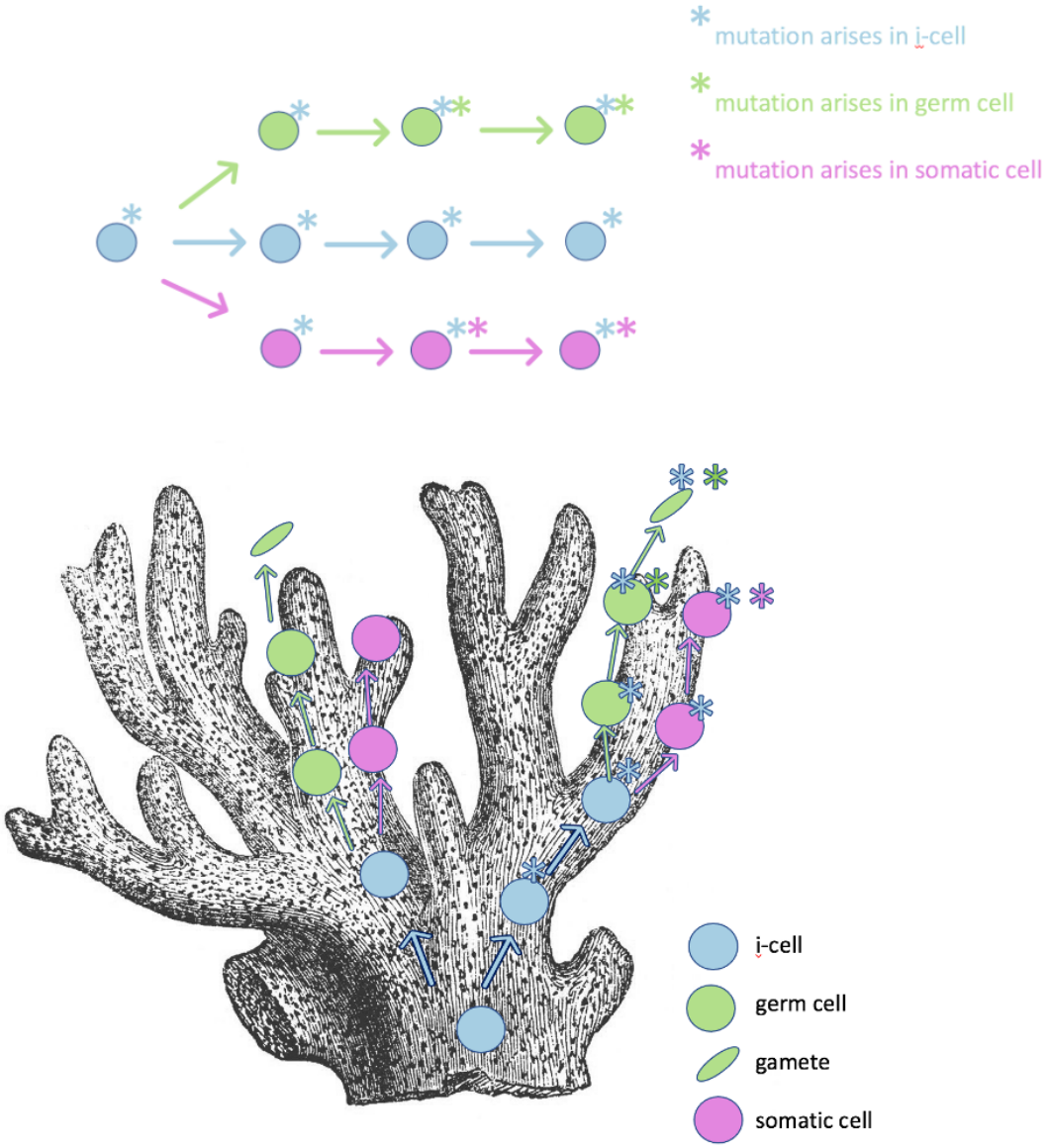


Figure 3. Schematics for how SNVs that arise at different stages of cell lineage development and differentiation proliferate. a.) SNVs that arise in i-cell may be found in both germ and somatic cells later, if the mutant i-cell lineage differentiates into both germ and soma. SNVs that arise in soma or germ cell lineage post-differentiation will only be found in those differentiated lineages. b.) The framework laid out in a) overlaid onto a branching, colonial coral structure suggests how SNVs in one branch of the colony may not be present in other branches.

Methods

Sample collection

279 Gravid coral colonies of *Acropora hyacinthus* were collected in Palau (Bureau of Marine
280 Resources permit number RE-19-07 and CITES permit PW19-018) in February 2019 and
281 transported to the Coral Spawning Lab at the California Academy of Sciences where they were
282 kept on a Palauan lunar and day/night cycle until spawning, with methods adapted from [24].
283 Colonies were monitored for spawning activity on nights 6 – 9 after the simulated full moon in
284 March 2019 (from 27 March to 30 March 2019). Prior to spawning, pliers were used to break off
285 2-3 cm branches that were "set," or showed visual signs of impending gamete release: three
286 branches from each of two colonies, and four branches from a third. Each branch was placed in a
287 labeled 5 mL vial of seawater where they spawned approximately 20 minutes later (Fig. 1a).
288 After the gamete bundles were released, they were transferred to labeled 1.5 mL tubes and left to
289 dissociate into eggs and sperm. Upon dissociation, eggs were removed via pipet, leaving a
290 concentrated sperm pool. Each concentrated sperm pool was pipetted into a 1.5 mL tube of
291 RNAlater. Each coral branch was placed in a 5 mL tube of RNAlater. Sperm pools in RNAlater
292 were stored at -20° C and coral branches in RNAlater were stored at -80° C until time for DNA
293 extraction.

294 *DNA Extraction and Library Preparation*

295 For each coral branch, the top layer of tissue was scraped from the coral skeleton with a
296 razor blade. DNA was then extracted from tissue using the NucleoSpin Tissue Mini kit columns
297 and corresponding protocol for extraction from animal tissue (Macherey-Nagel, Duren,
298 Germany). For each sperm pool, the tube containing RNAlater and sperm was vortexed
299 vigorously, then 200-400 ul of the sperm solution was pipetted out and mixed with 2x volume of
300 D.I. water. The sperm pools were then centrifuged for 3 minutes at 13,000 rpm. The supernatant
301 was pipetted off, leaving just the pelleted sperm at the bottom of the tube. DNA was extracted

302 from sperm pellets using the same Macherey-Nagel NucleoSpin Tissue Mini kit columns and
303 protocol as the parent tissue. Nextera full genome libraries were generated using a modified,
304 low-volume protocol optimized for coral DNA (Supplementary Methods). We constructed two
305 replicate libraries for each DNA extraction (Fig. 1b). Libraries were sequenced first on an iSeq
306 100 for quality control and then on a NovaSeq 6000 S4 at the Chan-Zuckerberg Biohub
307 Sequencing facility in San Francisco, CA, USA.

308 *Reference genome assembly*

309 In May 2020 we collected sperm from an additional *Acropora hyacinthus* colony for the
310 construction of a high quality *Acropora hyacinthus* reference genome assembly. This colony
311 originated in Palau and spawned at the California Academy of Sciences, where the sperm was
312 collected. Sperm was collected by pipetting, then it was rinsed and spun in seawater 3 times at
313 13,000 rpm for 3 minutes each spin (following methods from [25]). The cleaned sperm pellet was
314 then flash frozen in liquid nitrogen. The frozen sperm pellet was shipped to Dovetail Genomics
315 (Scott's Valley, CA, USA) for DNA extraction, sequencing, and genome assembly. The initial *de*
316 *novo* assembly was produced through a combination of Illumina short-read sequencing and
317 PacBio long-read sequencing. Proximity ligation was achieved with Dovetail™ Omni-C™
318 Technology, which uses a sequence-independent endonuclease approach to chromatin
319 fragmentation. The final genome assembly is made up of 908 scaffolds, of which 14 represent
320 full chromosome-length scaffolds, the same number of chromosomes as is in the *Acropora*
321 *millepora* genome [26]. The complete assembly is 446,422,234 nucleotides, with N_{50} = 26,527,962
322 nucleotides.

323 *Reference genome annotation*

324 Genome annotation was performed using MAKER2[27] in a *de novo*, iterative approach
325 based on https://gist.github.com/darencard/bb1001ac1532dd4225b030cf0cd61ce2.
326 Transcriptome evidence from *Acropora hyacinthus*[28] (and https://matzlab.weebly.com/data--
327 code.html), *Acropora millepora*[26,29], and *Acropora tenuis* (https://matzlab.weebly.com/data--
328 code.html) was provided for the initial round of annotation. Additionally, proteome evidence
329 from *Acropora digitifera*[30] and *Acropora millepora*[26] was utilized for the first round. Genome
330 wide repeat families were annotated by RepeatModeler2.0.1[31] and used as evidence for the initial
331 round. The *ab initio* gene predictors AUGUSTUS v3.2.3[32] and SNAP[33] were trained with the
332 gene models annotated by the previous round of annotation. The second round was then
333 conducted with these trained prediction models along with repeat, transcript, and protein
334 evidence annotated during the previous round. A third round of annotation was then performed
335 following the same procedures as round two. Following the final round, the completeness and
336 quality of the annotated transcriptome was assessed with BUSCOv5[34] and the OrthoDB v10[35]
337 eukaryota and metazoan datasets. The BUSCO score against the metazoan dataset was 71.3%
338 complete, 13.6% fragmented, and 15.1% missing (Supp. Table 3).
339 *Read mapping and SNP calling*
340 Adapters were trimmed from reads using Trimmomatic version 0.39. Trimmed reads
341 were mapped to the *Acropora hyacinthus* v1 genome using hisat2 with the parameters --very-
342 sensitive --no-spliced-alignment. Duplicate reads were removed with Picardtools
343 MarkDuplicates. Haplotype calling was performed with the Genome Analysis Toolkit version
344 4.1.0.0 Haplotypecaller tool[36]. We combined GVCFS from the same coral colony into a multi-
345 sample GVCF using CombineGVCFs. Joint genotype calling was then performed on each mutli-
346 sample GVCF using GenotypeGVCFs with the option –all-sites to produce genotypes for both

347 variant and nonvariants sites[37]. The genotype-called multi-sample VCFs were filtered with
348 SelectVariants to filter files by depth, with minimum depth and maximum depth determined by a
349 Poisson distribution of the average depth for a given sample, with $p < 0.0001$[38]. The filtered files
350 resulting from these steps were considered the "callable" regions of the genome, and were used
351 as the denominator for mutation frequency calculations. We filtered for just biallelic single
352 nucleotide polymorphisms (SNPs) using VCFtools. For the complete read mapping and SNP
353 calling pipeline see **https://github.com/eloralopez/CoralGermline**

354 *Identifying post-embryonic single nucleotide variants (SNVs)*

355 Single nucleotide variants from the genotyped colony VCFs using custom Python3 and R
356 scripts (**https://github.com/eloralopez/CoralGermline**). Putative post-embryonic SNVs were
357 identified by comparing the parent branch genotype calls from a given colony. A SNP was called
358 a putative post-embryonic SNV if the SNP a.) appeared in just one branch of the colony, and b.)
359 the SNP had the same genotype call in both replicate libraries from that mutant branch (Fig 1c,
360 d).

361 Germline mutations were identified by comparing the sperm genotype calls from a given
362 colony. A SNP was called a putative unique germline mutation if the SNP a.) appeared in just
363 one sperm pool spawned by the colony, b.) the SNP had the same genotype call in both replicate
364 libraries from the sperm pool, and c.) the genotype in the mutant sperm pool did not match the
365 genotype of the parent branch that spawned it (Fig. 1e). Alternatively, we called a SNP a
366 putative global germline mutation if the SNP a.) appeared in every replicate library from every
367 sperm pool spawned by the colony and b.) the genotype in the sperm pools did not match the
368 genotypes of any of the parent branches in that colony (Fig. 1f).

369 *Classifying putative SNVs*

370 Once we had generated a set of putative somatic and germline mutations, we classified
371 each SNV as either a Gain of Heterozygosity (GoH) or Loss of Heterozygosity (LoH) mutation,
372 and classified the directionality of the change (A to T, etc.) as described in [14].

373 *Final filtering of putative mutations to arrive at final set of mutations*

374 The final filtering step was to eliminate putative mutations that had been classified GoH
375 if the putatively mutant allele had been seen before in any of the other libraries. This step was
376 necessary because the filtering threshold to call a heterozygotes was 10%. This means that if the
377 putatively mutant allele was present at <10% allele frequency in other samples, it was not truly a
378 mutant, but present in the colony at low levels. Similar, we eliminated putative mutations
379 classified as LoH if the mutant sample was heterozygous at a level <10%. The mutations that
380 passed this filter is the final set of mutations upon which all subsequent analyses were
381 performed.

382 *Are parent SNVs shared by their sperm pool or not?*

383 Once we arrived at a set of somatic mutation with all filters applied, we checked to see if
384 the genotype of the mutant parent matched the genotype of the sperm pool that came from that
385 branch. GoH SNVs were considered SHARED if one or both genotypes of the two sperm pool
386 replicates spawned by the mutant branch matched the mutant genotype and NOT SHARED if
387 neither of the genotypes of the two sperm pool replicates spawned by the mutant branch matched
388 the mutant genotype (Fig. 1c, d). LoH SNVs were considered SHARED if both genotypes of the
389 two sperm pool replicates spawned by the mutant branch matched the mutant genotype and NOT
390 SHARED if one or neither of the genotypes of the two sperm pool replicates spawned by the
391 mutant branch matched the mutant genotype (Fig. 1c, d).

392 *Designating SNV effects on codons*

393 We classified each mutation by the genomic region (intron, exon, etc.) it fell in and, if it
394 fell in a coding region, the type (synonymous, missense) using the program snpEff[39] configured
395 with the *Acropora hyacinthus* version 1 genome. We calculated the rate of missense and
396 synonymous SNVs per bp in the coding region by dividing the number of SNVs by the total
397 callable coding region. We calculated the average percent of coding SNVs that were missense
398 per sample for three categories: Parent Only, Parent and Sperm, and Single Sperm Pool Only.
399 There were no coding SNVs in the All Sperm Pools category (Extended Data Fig. 6).

400 *Selection on mutations*

401 We calculated dN/dS, a measure of selection on the genome, using the R package
402 *dndscv*[21]. We first created a reference CDS file for the *Acropora hyacinthus* genome using the
403 *Acroporal hyacinthus* genome annotations (gff3) file[26] and a custom Python3 script. We then
404 created *dndscv* input files from the filtered set of mutations found in each coral colony. We ran
405 *dndscv* with theta = 0.

406 *Mutation rates*

407 To catalogue post-embryonic SNVs, we compared sequences from 3 parent branches in
408 each colony and recorded cases in which one branch showed a genotype different from all the
409 others. This catalogue includes true somatic mutations in that branch, but also includes any
410 sequencing or PCR errors injected during sample preparation and sequencing. To limit these
411 errors, we compared sequences from each technical replicate and catalogued a mutation only
412 when it was visible in both replicates of a branch and in none of the replicates of any other
413 branch. Comparing technical replicates was highly successful at reducing noise from sequencing
414 error, eliminating over 90% of putative SNVs that would have been identified using the same
415 pipeline and filters without technical replicates (Extended Data Figs. 2, 3). To find the average

416 frequency per nucleotide of somatic mutations unique to a given branch in a coral colony, we
417 divided the number of SNVs in a sample by the number of total callable nucleotides sequenced
418 for that sample (Supplementary Table 1).

419 $$\text{\# SNV per Mb} = (\text{\#SNVs / Callable region (bp)}) * 1{,}000{,}000$$

420 The callable genome sizes were 1.2×10^8 bp, 1.2×10^8 bp, and 0.80×10^8 bp and the
421 callable coding region sizes were 1.3×10^7 bp, 1.3×10^7 bp, and 0.91×10^7 for each of the three
422 colonies, CA56, CA60, and CA65, respectively (Supp. Table 1).

423 *Tissue mixtures*

424 Because DNA was extracted from tissue scrapings encompassing multiple polyps per
425 parent branch, we were initially concerned that the parent sample might be a heterogenous mix
426 of somatic and germ tissues. Germ and stem cells in other cnidarians tend to reside at the base of
427 the polyp, so the scraping method was intended to take off just the somatic tissue and not the
428 germ or stem cells. To check this, we plotted the average variant allele frequency (VAF) of the
429 mutant parent (that is, the average VAF for the two technical replicate libraries) against the
430 average VAF of the mutant sperm pool.

431 $$\text{VAF} = \text{\# of reads supporting SNV / total \# of reads at locus}$$

432 $$\text{Average VAF} = (\text{VAF}_1 + \text{VAF}_2)/2$$

433 If the parent samples were a mix of somatic and germ and/or stem cells, then a germline
434 mutation may have erroneously been called a somatic mutation. If that were the case, then the
435 VAF of the parent would be considerably lower than the VAF of the sperm pool, because the
436 sperm pool's mutant would not be diluted by nonmutant somatic tissue. In that case, we would
437 expect the trendline of the parent VAF: sperm VAF linear model to have a slope significantly
438 greater than 1. In reality, the slope of the trendline was less than 1, suggesting that we

439 undercounted the number of mutant reads in the sperm. This gives us confidence that the parent
440 samples were in fact all or almost all somatic tissue, and that the mutations found in the parents
441 were in the soma. We used a linear regression model to check the relationship between the
442 variant allele frequency of the mutant parent and the variant allele frequency of the mutant sperm
443 for all inherited GoH mutations, where variant allele frequency equals the number of reads with
444 mutation divided by the total number of reads. The slope of the trendline was 0.36, with R = 0.3
445 and p = 5.9e-08 (Extended Data Fig. 7). This suggests that there may have been some
446 undercounting of the number of mutant reads in the sperm. The distribution of average VAF in
447 the parents for SNVs classified as "Parent Only" has a larger leftward skew, which also suggests
448 that some SNVs found at low frequency in the parent samples may have been missed in the
449 sperm (Extended Data Fig. 8). This gives us confidence that the parent samples were in fact all
450 or almost all somatic tissue, and that the mutations found in the parents were in the soma.

451 *Types of mutations*

452 For Gain of Heterozygote mutations (where the mutant genotype is a novel heterozygous
453 SNP) we classified the somatic mutation as inherited by the sperm if at least one of the technical
454 replicate libraries of the sperm contained the mutant allele. If neither sperm library contained the
455 mutant allele, then it was considered not inherited by the sperm. For Loss of Heterozygosity
456 mutations (where the mutant is a homozygous genotype at a site for which the parent colony is
457 heterozygous) to be classified as inherited by the sperm both sperm libraries had to be entirely
458 homozygous for the allele of the mutant parent. For these cases, if any read for a parent sample
459 showed the minor allele also seen in the putative GoH mutation, we did not call these as
460 mutations. Likewise, if any of the sperm reads showed the minor allele also seen in the parent

branch, we did not call this a LoH mutation. These stringent filters reduced the number of mutations we could characterize but led to high confidence in our characterization of variants.

Data availability

All raw fastq files, as well as the *Acropora hyacinthus* genome version 1 assembly, are accessioned under BioProject PRJNA707502 at NCBI. The accession numbers for the fastqs are SAMN18207983-SAMN18208014 and the accession number for the assembly FASTA is SAMN20335437.

Code availability

The code used for this study can be found at **https://github.com/eloralopez/CoralGermline**.

Methods References

24. Craggs, J. *et al.* Inducing broadcast coral spawning ex situ: Closed system mesocosm design and husbandry protocol. *Ecol. Evol.* **7**, 11066–11078 (2017).
25. Shumaker, A. *et al.* Genome analysis of the rice coral Montipora capitata. *Sci. Rep.* **9**, 1–16 (2019).
26. Fuller, Z. L. *et al.* Population genetics of the coroal Acropora millepora: Toward genomic prediction of bleaching. *Science (80-.).* **369**, (2020).
27. Holt, C. & Yandell, M. MAKER2: An annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics* **12**, 1–14 (2011).
28. Barshis, D. J. *et al.* Genomic basis for coral resilience to climate change. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 1387–92 (2013).
29. Moya, A. *et al.* Whole Transcriptome Analysis of the Coral Acropora millepora Reveals Complex Responses to CO 2-driven Acidification during the Initiation of Calcification.

484 *Mol. Ecol.* **21**, 2440–2454 (2012).

485 30. Shinzato, C. *et al.* Using the Acropora digitifera genome to understand coral responses to
486 environmental change. (2011). doi:10.1038/nature10249

487 31. Flynn, J. M. *et al.* RepeatModeler2 for automated genomic discovery of transposable
488 element families. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 9451–9457 (2020).

489 32. Stanke, M. & Waack, S. Gene prediction with a hidden Markov model and a new intron
490 submodel. in *Bioinformatics* **19**, 215–225 (Oxford Academic, 2003).

491 33. Korf, I. Gene finding in novel genomes. *BMC Bioinformatics* **5**, 1–9 (2004).

492 34. Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. M.
493 BUSCO: Assessing genome assembly and annotation completeness with single-copy
494 orthologs. *Bioinformatics* **31**, 3210–3212 (2015).

495 35. Kriventseva, E. V. *et al.* OrthoDB v10: Sampling the diversity of animal, plant, fungal,
496 protist, bacterial and viral genomes for evolutionary and functional annotations of
497 orthologs. *Nucleic Acids Res.* **47**, D807–D811 (2019).

498 36. Poplin, R. *et al.* Scaling accurate genetic variant discovery to tens of thousands of
499 samples. *bioRxiv* 201178 (2017). doi:10.1101/201178

500 37. Van der Auwera, G. A. *et al.* From fastQ data to high-confidence variant calls: The
501 genome analysis toolkit best practices pipeline. *Curr. Protoc. Bioinforma.* **43**, 11.10.1-
502 11.10.33 (2013).

503 38. Wu, F. L. *et al.* A comparison of humans and baboons suggests germline mutation rates
504 do not track cell divisions. *PLoS Biol.* **18**, e3000838 (2020).

505 39. Cingolani, P. *et al.* A program for annotating and predicting the effects of single
506 nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain
507 w1118; iso-2; iso-3. *Fly (Austin).* **6**, 80–92 (2012).

508 **Acknowledgements**

509 EHLN was funded by NSF GRFP and a Stanford Graduate Fellowship in Science and
510 Engineering (Morgridge Family Foundation). Research funded by Chan-Zuckerberg BioHub,
511 NSF OCE-1736736, and the Hope for Reefs Initiative. We thank N. Neff at CZ-BioHub for
512 sequencing power and A. Voskoboynik, P. Bump, and B. Cornwell for stem cell insights. Thanks
513 to R. Ritson-Williams, R. Ross, and BIOTA for field collection support, and thanks to the staff
514 of the Steinhart Aquarium for taking care of the corals.

515 **Author contributions**

516 EHLN, RA, and SRP designed the study. RA kept corals alive and healthy and facilitated lab
517 spawning. EHLN and RA collected parent and sperm samples for this study. EAS optimized the
518 library preparation protocol used in this study. EHLN extracted DNA and constructed libraries
519 for all samples. EHLN designed and performed all bioinformatic analyses for the study, except
520 for genome annotation, which was performed by EAH. EHLN wrote the manuscript and all
521 authors provided input and feedback on drafts.

522 **Competing interest declaration**

523 The authors have no competing interests to declare.

524 **Additional Information**

525 Supplementary information is available for this paper:

526 Supplementary Table 1. Summary of sequencing depth and SNV counts (.txt file)

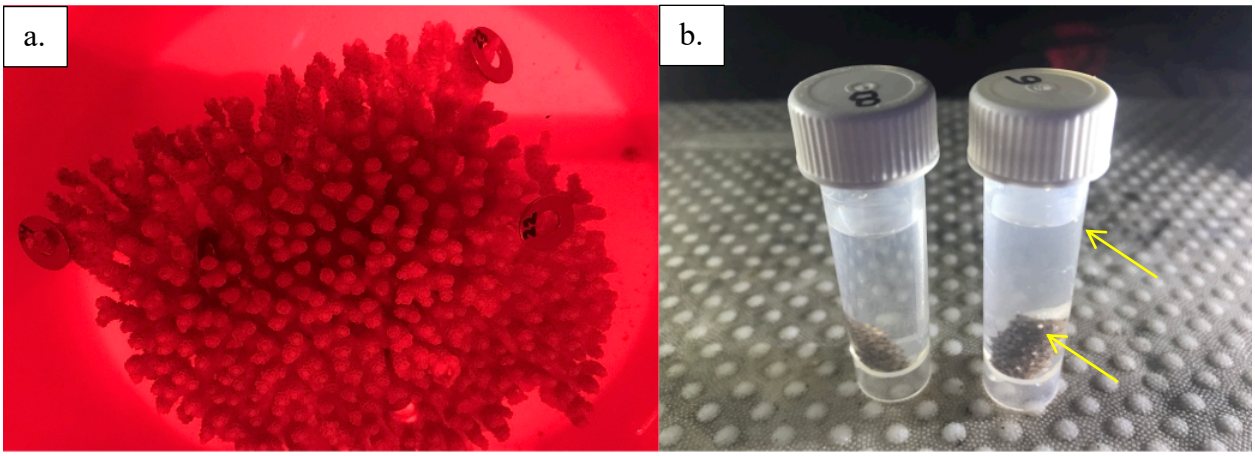
527 Supplementary Table 2. List of verified SNVs and their metadata (.txt file)

Supplementary Table 3. Summary statistics for *Acropora hyacinthus* version 1 genome annotations (.xlsx file)

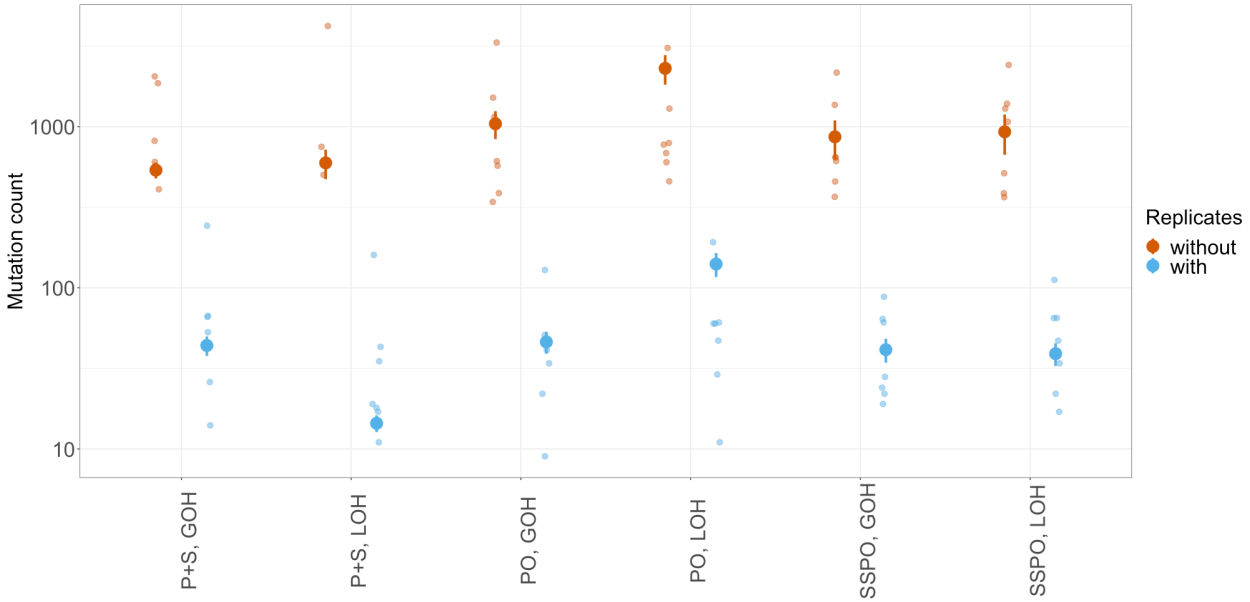
Supplementary Methods. The optimized full genome library preparation protocol for corals (.docx file).

Correspondence and requests for materials should be addressed to Elora H. López-Nandam, elopez-nandam@calacademy.org

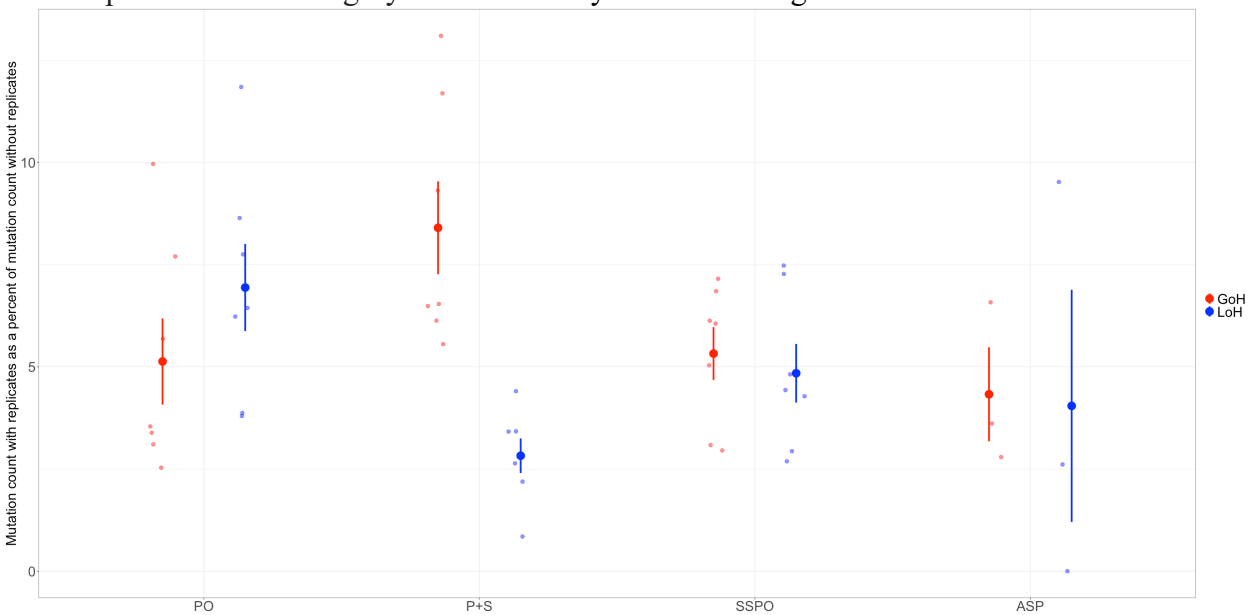
Extended data figure/table legends



Extended Data Figure 1. Photos of the experimental setup. a.) Colony 60, with sampled branches denoted by numbered washers. For scale, the outside diameter of each washer is 1.59 cm. b.) Two of the branches (CAP6 and CAP8) sampled from Colony 56, releasing gamete bundles (indicated by arrows) into their respective 5 ml tubes of seawater.

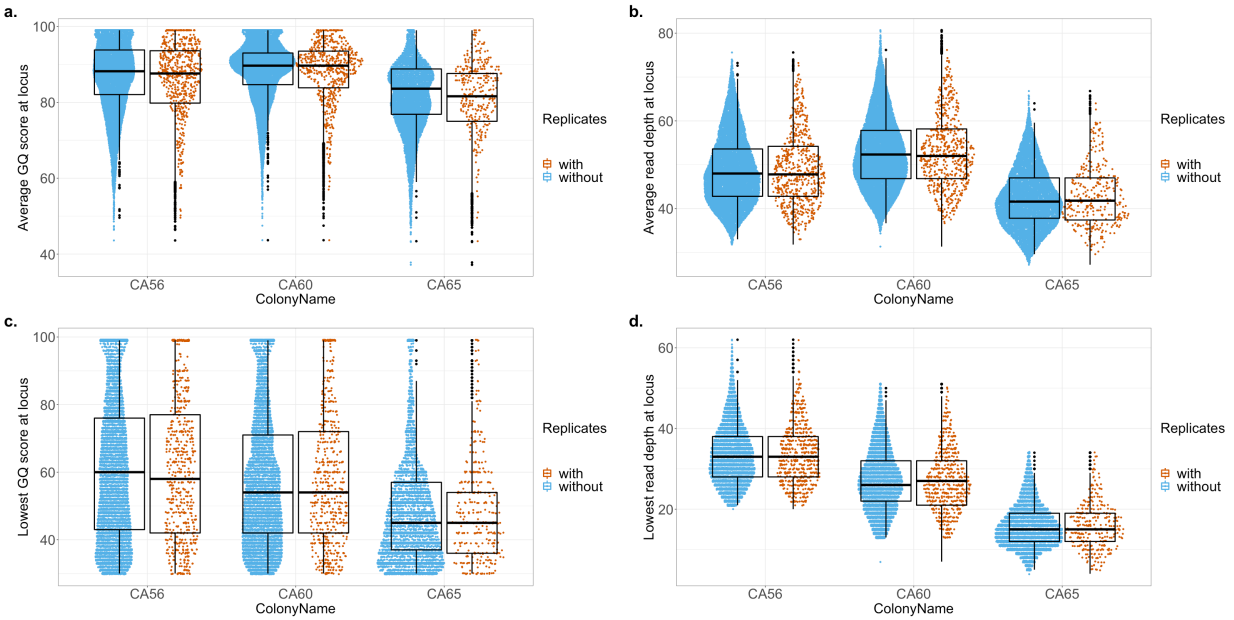


Extended Data Figure 2. The mean number of mutations identified in each parent branch in the Parent Only (PO) or Parent and Sperm (PO), or single sperm pool only (SSPO) when looking at one library per sample (aka without technical replicates, pink) or two libraries per sample (with technical replicates, gray). Error bars represent ± 1 s.e.m. Each data point (N=7) is shown as a smaller point for each category. Note that the y axis is on a log-10 scale.

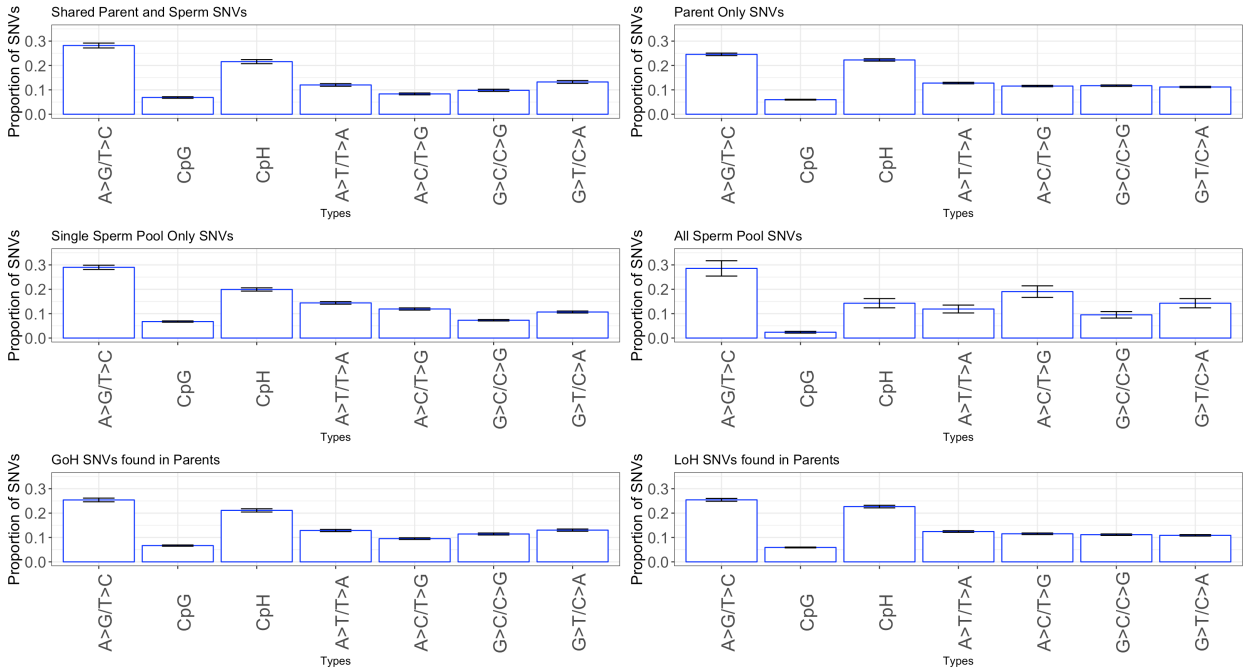


Extended Data Figure 3. The percentage of mutations identified when replicate libraries are included for each of the four categories of SNVs: Parent Only (PO), Parent and Sperm (P+S), Single Sperm Pool Only (SSPO), and All Sperm Pools (ASP). Error bars represent ± 1 s.e.m. Each data point (N=7) is shown as a smaller point for each category. The percentage is expressed as a fraction of the number of mutations identified when no technical replicates are included, such that:

% = (# of mutations identified using technical replicates*100) / (# of mutations identified without technical replicates)



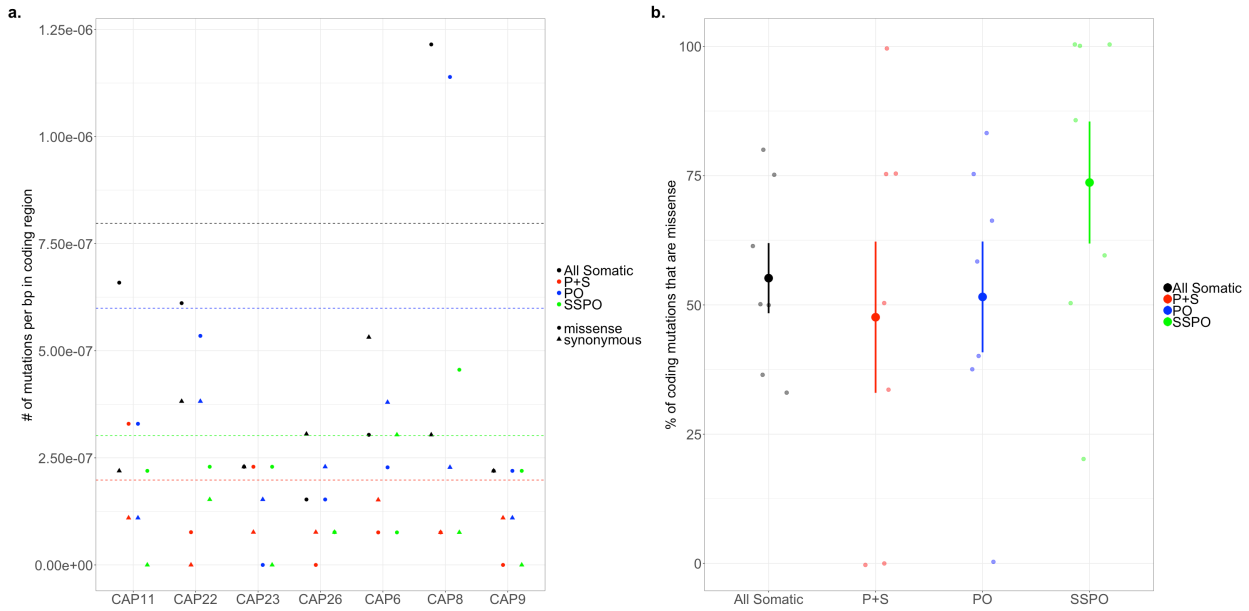
Extended Data Figure 4. Distribution of average GQ score and average read depth for each mutation in the colony (top) as well as distributions of the lowest GQ score and lowest read depth for every mutation identified in each colony (bottom). Mutations found when using no technical replicates are shown in pink, and the mutations found when including technical replicates shown in gray.



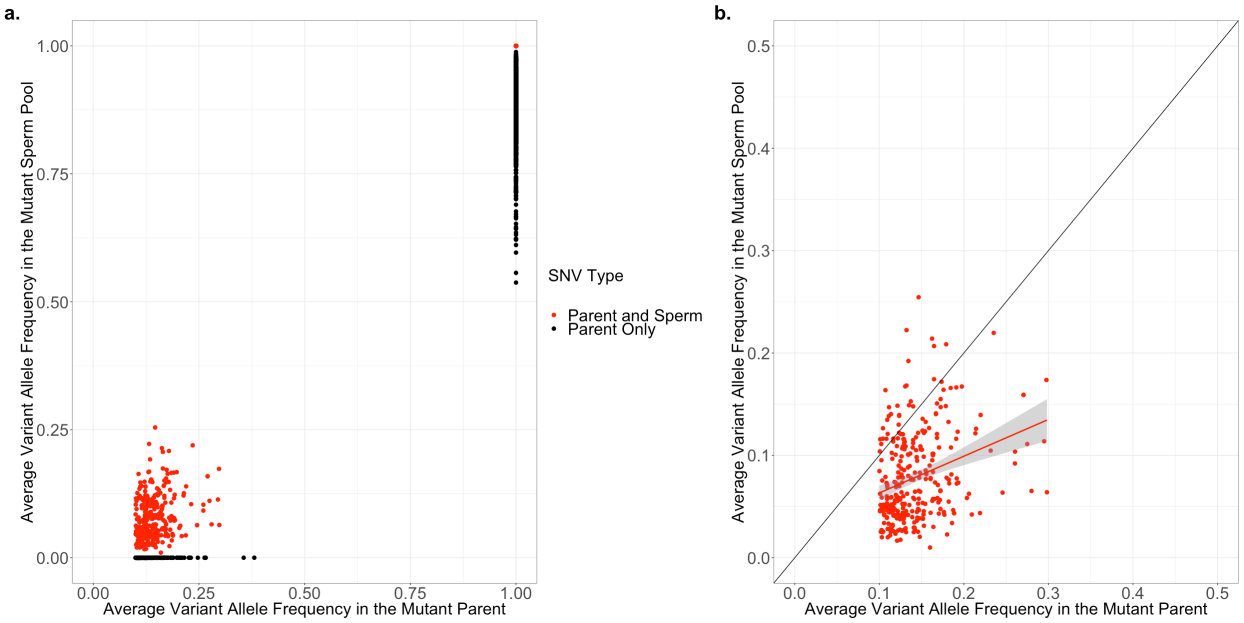
Extended Data Figure 5. SNV spectra from various subsets of SNVs. None of the SNV spectra were significantly different each other for the different data subsets (see X^2 tests below). Error bars represent ± 1 s.e.m.

X^2 tests of independence results for Extended Data Figure 5:

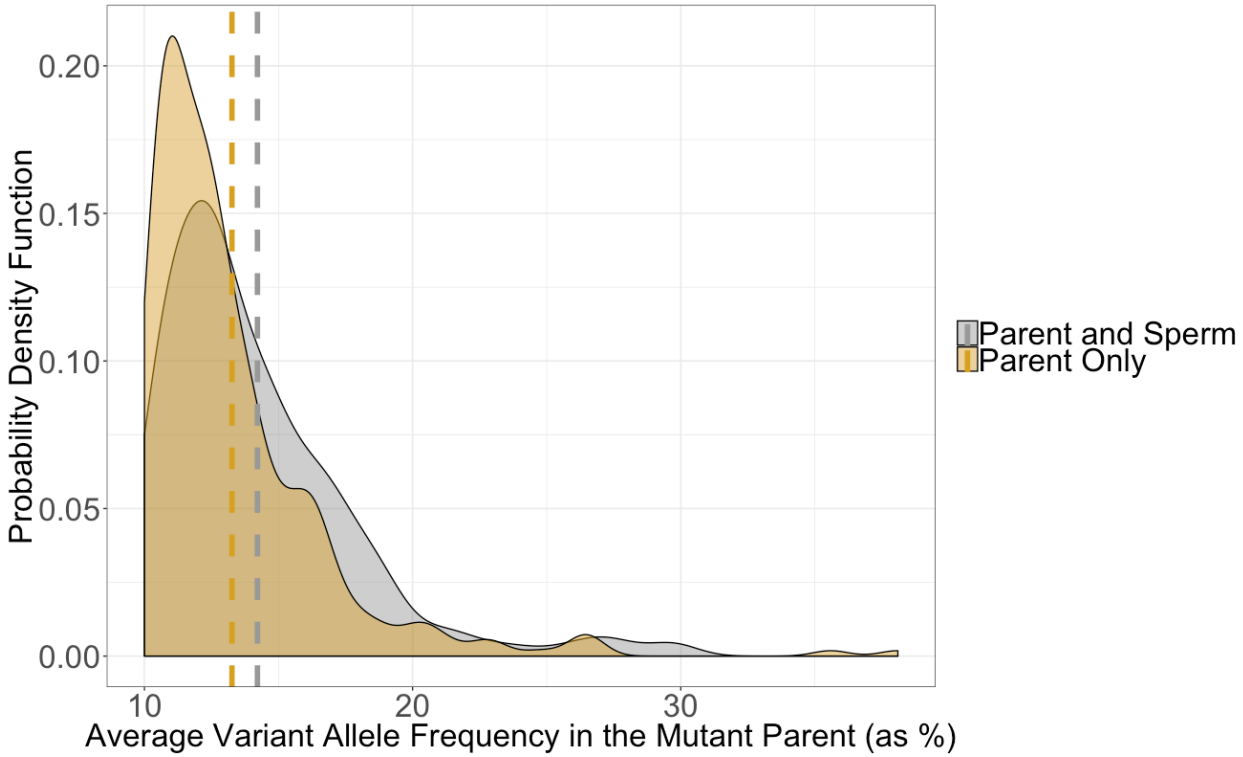
Comparison	X^2
Parent only vs. Shared Parent and Sperm	df = 6, N=1715, X^2 = 7.3495, p= 0.2897
Single Sperm Pool Only vs. All Sperm Pools	df = 6, N=604, X^2 = 4.2981, p= 0.6364
Parent GOH SNVs vs Parent LOH SNVs	df = 6, N=1715, X^2 = 3.9411 p= 0.6846



Extended Data Figure 6. Rates and percentages of missense and synonymous mutations across sample and category. a.) The number of missense (circle) and synonymous (triangle) SNVs per bp of the coding region for each category (all somatic, P+S, PO, and SSPO) for each sample. Dashed lines indicate the mean number of coding SNVs (the sum of missense and synonymous) for each category across all samples. b.) The mean percent missense for each category, with error bars indicating ± 1 s.e.m. Each data point (N=7) is shown as a smaller point for each category.



Extended Data Figure 7. Variant allele frequencies for parents and their respective sperm pools. a.) GOH mutations for which sperm VAF =0 were classified as Parent Only (black), likewise LOH variants in the parent for which the VAF < 1 in the sperm were classified as Parent Only. All other SNVs were classified as being shared by both a parent branch and its corresponding sperm pool (red). B.) For GOH SNVs that are shared by a parent branch and its sperm, average variant allele frequency of the two replicate parent libraries is positively correlated with the average variant allele frequency of the two replicate sperm pool libraries. The slope of the relationship is 0.36 and R=0.3 (red line). The black line shows a 1:1 line for reference.



Extended Data Figure 8. The distribution of variant allele frequencies in the parent branches for GOH SNVs in the parents only (orange) and in the parent and sperm samples (gray). The mean parent variant allele frequency for each category is marked by dashed lines.

Extended Data Table 1. Comparison of SNV frequencies in the coding region vs. across the full genome for six SNV types (shown in Figure 2c). Error shown is ±1 s.e.m.

Type	Average frequency per bp (Full genome, coding)	Wilcox test (two-tailed, paired)
Parent Only, GOH	Full: $4.4 \times 10^{-7} \pm 0.6 \times 10^{-7}$ Coding: $9.2 \times 10^{-8} \pm 3.1 \times 10^{-8}$	V=28, P= 0.016
Parent Only, LOH	Full: $1.32 \times 10^{-6} \pm 0.19 \times 10^{-6}$ Coding: $5.2 \times 10^{-7} \pm 1.4 \times 10^{-7}$	V=28, P= 0.016
Parent and Sperm, GOH	Full: $4.8 \times 10^{-7} \pm 0.9 \times 10^{-7}$ Coding: $1.8 \times 10^{-7} \pm 0.6 \times 10^{-7}$	V=27, P= 0.031
Parent and Sperm, LOH	Full: $2.6 \times 10^{-7} \pm 1.1 \times 10^{-7}$	V=28, P= 0.016

	Coding: $1.6 \times 10^{-8} \pm 1.6 \times 10^{-8}$	
Single Sperm Pool Only, GOH	Full: $3.8 \times 10^{-7} \pm 0.5 \times 10^{-7}$ Coding: $1.5 \times 10^{-7} \pm 0.5 \times 10^{-7}$	V=28, P= 0.016
Single Sperm Pool Only, LOH	Full: $3.6 \times 10^{-7} \pm 0.4 \times 10^{-7}$ Coding: $1.6 \times 10^{-7} \pm 0.6 \times 10^{-7}$	V=27, P= 0.031