Parental care drives the evolution of molecular genetic variation

Mashoodh R*, Trowsdale A, Manica A, Kilner RM

Department of Zoology, University of Cambridge, United Kingdom

*Corresponding author

Email: rm786@cam.ac.uk

Abstract

Whilst genes can drive social traits, social traits themselves can create and maintain genetic variation in populations. The resulting underlying genetic variation can shape how individuals respond to challenges (e.g., stress, undernutrition) and/or predict how rapidly populations adapt to changing environments (e.g, climate change, habitat destruction). Here, we investigate how a social behaviour, parental care, can shape molecular genetic variation in the subsocial insect *Nicrophorus vespilloides*. Using whole genome sequencing of populations that have evolved in the presence or absence of parental care for 30 generations, we show that parental care can increase levels of standing genetic variation. In contrast, under a harsh environment without care, strong directional selection causes a reduction in genetic variation. Furthermore, we show that adaptation to the loss of care is associated with genetic divergence between populations at loci related to stress, morphological development and transcriptional regulation. These data shed light on the genetic processes that shape and maintain genetic diversity in response to parental care within populations and the mechanisms of adaptation to stressors in the face of the extreme loss of care.

Introduction

While much attention has been paid to identifying genes that drive social behaviours [1,2], relatively few studies have focused on how changes in social behaviour could affect the accumulation and maintenance of genetic variation. Yet, social living is associated with large-scale restructuring and the evolution of genome organization and architecture [3]. In humans, benevolent social activities, such as modern health care, are thought to have led to the accumulation of deleterious mutations within populations [4,5]. Moreover, the capacity for co-operative brood care in insect societies to maintain and/or buffer against extreme changes in effective population size is thought to be a key determinant of population stability [6–8]. Therefore, the extent to which genetic variation is affected by social behaviour has implications for the health of populations and their capacity to rapidly adapt to environmental perturbations. Here we investigate how a cooperative social behaviour, namely the supply of parental care, contributes to genome-wide levels of genetic variation. We focus on parental care in a subsocial pair-breeding insect, rather than more elaborate forms of sociality, to avoid the confounding effects of extreme reproductive skew on genetic variation, which is common in cooperative insect societies.

Cooperative social interactions often function to shield social partners from a harsh physical environment and the same is true for parental care [9,10]. Without cooperation generally, and care specifically, individuals would be exposed to strong, frequently directional, selection pressures from the abiotic environment, which would favour the evolution of new adaptations and cause an associated reduction in genetic variation. On the other hand, the presence of cooperative care relaxes selection from this wider environment, theoretically allowing genetic variation to accumulate. Indeed, several lines of evidence have shown that cooperative social behaviours, including care, are sufficient to allow mildly deleterious mutations to accumulate within populations [11–15]. In this way, parental care, could shift the 'mutation-selection' balance by relaxing selection and preventing the elimination of new spontaneous mutations. The resulting increase in genetic variation could emerge in the form of single nucleotide polymorphisms (SNPs) and/or other structural genetic variants (e.g., indels, transposable elements and/or recombination events) depending on the natural mutation rate of such variants. Such "cryptic" variants could then be maintained in the population with a combination of environmentally-induced genetic capacitors, epigenetic modifications and/or RNA-mediated signals [16]. Despite the widespread suggestion that changes in parental care can shape genetic variation, we still have a poor understanding of how and where such changes might occur on a molecular genetic level.

Here we use evolving populations of burying beetles (*N. vespilloides*) to explore how parental care affects levels of standing genetic variation and how populations may adapt in the face of its loss. In natural populations of this locally abundant subsocial insect, burying beetle parents raise their young on a carrion nest, formed from a small dead animal, such as a mouse or songbird. There is continuous variation in the level of parental care supplied, with some parents leaving before their young have even hatched [17]. We exploited this natural variation in care to

establish two types of experimentally evolving populations in the laboratory, which varied only in the family environment that larvae experienced during development, and where the same family environment was created for successive generations within populations. In Full Care populations (FC), parents remained with their young throughout development; whereas in No Care populations (NC), parents were removed just prior to hatching. We have previously shown that No Care populations evolved adaptively [18] and divergently from Full Care populations in the extent of the pre-hatching care behaviours [19], the extent of sibling cooperation [20,21] and in their larval morphology [22]. We use pooled whole-genome re-sequencing of these populations to document genetic variation at the molecular level when care was present and when it was prevented experimentally. First, we determined the effect of care on within-population genetic variation (SNP diversity; theta and pi). Second, we identified the genetic loci that had diverged to the greatest extent following the removal of care by looking for regions of high genetic differentiation (F_{ST}) between experimental populations.

Methods

Breeding design & Experimental Evolution

We sampled DNA from experimental populations of *Nicrophorus vespilloides* that had been evolving under different regimes of parental care, and which were founded from a genetically diverse founding population generated by interbreeding beetles from multiple wild populations across Cambridgeshire. These populations have been described in detail previously [18], and comprise a total of 4 populations: two blocks (Block 1 and Block 2; separated by 1 week) containing two populations evolving with (FC_{POP}) or without parental care (NC_{POP}). On the 29th generation, when individuals were sexually mature, we paired 15 males and females within each population (N=60 pairs in total). Each pair was placed in a separate breeding box with moist soil and a thawed carcass (10-12g). We then placed each breeding box in a cupboard and allowed parents to prepare the carcass and for the female to lay the clutch of eggs. After 53h, populations were split such that both parents were either removed (in keeping with the procedure experienced by the NC_{POP}). Approximately 80h after hatching we randomly selected 4 larvae from each family for DNA extraction.

Larval tissue dissection, DNA extraction & Whole-genome sequencing

For each family, DNA from heads of first instar larvae were pooled and extracted using a modified version of the Qiagen DNEasy Mini Kit. Total DNA quality was checked using gel electrophoresis and yield was quantified using a Qubit DNA Assay Kit (Thermo Fisher). Families were pooled in equimolar concentration to generate 4 libraries: FC1, FC2, NC1, and NC2 with pool sizes of 82, 104, 104, 118 individuals, respectively. Each family was represented equally within the sequencing

library. Whole genome re-sequencing libraries were constructed and sequenced (150bp paired-end) at a depth of 100x using an Illumina Novaseq 6000 platform by Novogene (Hong Kong).

Bioinformatic Analyses

Reads were trimmed using TrimGalore (0.5.0; https://github.com/FelixKrueger/TrimGalore) to remove adaptor sequences, perform quality trimming and discard low-quality reads. Reads were aligned in paired-end mode using the burrows-wheeler aligner (bwa) to the *N. vespilloides* reference genome (NCBI Refseq Assembly: GCF_001412225.1) [23,24]. See **Supplementary Table 1** for read mapping statistics. Duplicates were removed using PicardTools (http://broadinstitute.github.io/picard/) and indels were filtered using the Popoolation toolbox [25]. We used pi and theta to measure levels of standing genetic variation within populations. To measure the extent of standing genetic variation within populations, pi and theta statistics were calculated for non-overlapping 1000bp windows across the genome using tools from Popoolation. To measure the extent of genetic divergence between populations, we used Popoolation2 [26] to calculated the pairwise fixation index (F_{ST}) for all combinations of population pairs across 500bp sliding windows (250bp overlap) across the genome. SNPs were called using sites with read counts between 40 and 400. To find windows where evolving populations may have diverged, controlling for random drift, we computed a relative F_{ST} ratio of the geometric mean of the between-population comparisons (i.e., FC1;NC1, FC1;NC2, NC1;FC2, FC2;NC2) to the arithmetic mean of the within-population F_{ST}'s (FC1;FC2, NC1;NC2)); in other words, we focussed on windows with small differences between populations under the same selection regime but large differences in populations with different regimes. The top 1% of F_{ST} ratios were selected as regions of interest. Additionally, we extracted genome-wide F_{ST} using the R package *poolfstat* [27] and population structure of all SNPs using the core model of BayPass version 2.1 [28] to estimate scaled covariance (Ω) matrices. All subsequent statistical analyses were performed in *R* version 4.1.2 using the core *R stats* package [29]. Data wrangling and visualisations were performed using the *tidyverse* suite [30].

Functional Annotation

Functional enrichment analyses were conducted using the topGO R package version 2.38.1 [31] to identify over-representation of particular functional groups within the DEGs in response to the removal of care as well as the evolved response to the removal of care, based on GO classifications using Fisher's exact test. GO terms were annotated to the *N. vespilloides* genome using the BLAST2GO (version 5.1.1) workflow to assign homologs to the *Drosophila* non-redundant protein databases [32].

Data Access & Code Availability

All raw sequencing data generated have been submitted to the NCBI Gene Expression Omnibus (GEO) under accession number (#######). All code for the analyses contained within this manuscript can be found at: https://github.com/r-mashoodh/nves_dnaEvol.

Results

Standing genetic variation between populations

To determine if populations differed in the extent of standing genetic variation we commuted both theta (theta) and pi (symbol) statistics for each population across 1000bp non-overlapping windows. Populations that evolved under Full Care (FC1 and FC2) had higher theta values than populations evolved under No Care (NC1 and NC2) (Block 1: H(1)=59.21, *p*=1.418e-14; Block 2: H(1)=61.60, *p*=4.199e-15). Similarly, there were higher Pi values in FC compared to NC, though this effect was not present in Block 1 (Block 1: H(1)=3.45, p=0.06; Block 2: H(1)=159.8, *p*<2.2e-16). Together, these results suggest that FC populations maintained more SNP diversity compares to populations evolving under NC (**Table 1**). However, evolving populations did not differ in their mutation spectrum (**Supplementary Figure 1**).

Adaptive genetic differences between populations

Hierarchical clustering of populations was consistent with the idea that the NC populations have diverged from their FC counterpart as there was more shared variation within replicates than between (**Supplementary Figure 2**). Genome-wide pairwise F_{ST} differences indicated that genetic divergence exists between experimental (NC) populations as well as between control (FC) populations (**Supplementary Table 2**). To control for any random drift between populations, we used an F_{ST} of between and within population divergence to look for consistent divergence across the replicates (**Figure 1A**; see Methods). Using this approach we identified 331 differentiated genes (**Supplementary Table 3**), with 10 genes overlapping with the 5'UTR region (Figure 1?). These genes were generally enriched for GO processes associated with morphogenesis, neural development, immunity, and chromatin organization (**Figure 1B; Supplementary Table 4**).

Discussion

We found that populations with parental care had greater levels of genetic variation, in the form of higher theta and pi diversity, than in the populations where care was prevented. This suggests that presence of parental care can relax selection sufficiently to lead to the accumulation of SNP variants. The majority of this accumulated genetic variation is likely to be either neutral or mildly deleterious, since the majority of new mutations generally fall into either of these two categories [5,33]. Indeed, we have previously demonstrated that inbreeding of these populations resulted in faster extinction of Full Care compared to No Care populations, further suggesting that, at least some, of the variation accumulated in the presence of care was deleterious [14]. Although we measured only SNP variation here, genetic variants that arise through different types of

mutation or recombination could also, in theory, be maintained in the population by parental care. Whether care specifically favours particular mutants remains to be tested in future studies.

In contrast, the harsher No Care environment imposed strong directional selection resulting in rapid adaptation [18,21] and reduced levels of standing genetic variation. These results are highly suggestive of adaptation in the laboratory populations from standing genetic variation already present in the founding wild populations who are inclined to provide care. The loss of care in *N. vespilloides* is likely to be associated with greater levels of environmental stress during development and heightened exposure to pathogens from the carrion resource [34,35]. We have previously shown that adaptation to a No Care environment is associated with gene expression signatures that show blunted stress responses and compensatory expression in metabolic and developmental pathways [34]. Our data here suggest that adaptation caused the No Care and Full Care populations to diverge genetically at loci that could promote immunity, metabolic and behavioural stress resilience in the absence of care.

We have previously shown that larvae from the No Care populations evolved to show greater levels of sibling cooperation than larvae from the Full Care populations [15,20]. Our finding of divergence between the populations at the cytochrome P450 gene, which appears to be a homolog of the Drosophila *Cyp6a20* gene, is therefore of particular interest since deletions of this gene have been previously associated with higher levels of aggression and reduced sociality in fruit flies [36]. Furthermore, this gene is also intertwined with juvenile hormone pathways which are known to be involved in multiple facets of behavioural and morphological development [37] and could facilitate better access to, or use of, the carrion breeding resource. Not surprisingly, many of the genetic differences between the populations are in upstream regions and/or genes that encode for transcription factors or chromatin modifiers, suggesting that changes in regulatory function is a key component of adaptation to the loss of care. Differences in regulatory functions could shape levels of gene expression of other genes, further buffering against stress in the absence of parental care [34]. Although we cannot identify a single gene or master regulator within the genetic changes, these data identify candidate genes that might play key roles in conferring resilience to the loss of care, and environmental stressors more broadly.

While we show here that parental care contributes to genetic variation through its effect on selection, it is possible that the incidence of mutation is itself reduced by the loss of parental care. Mutation rates have a strong genetic basis and can vary between individuals and amongst populations [38,39]. Given that the loss of care is a major developmental stressor, and that stress has been shown to induce mutations, adaptation to the loss of care could involve genetic mechanisms that dampen and/or buffer the consequences of new mutations that arise [11,33]. Consistent with this hypothesis, we found high levels of genetic differentiation amongst genes involved in DNA replication and repair (e.g., flap endonuclease 1, FEN1, and the checkpoint protein, HUS1). Though we did not detect major biases in the mutation spectrum between populations, these genes could facilitate efficient DNA repair and further shape the mutation rate.

In short, each populations could favour an optimal mutation-selection balance, resulting in different levels of standing genetic variation based on levels of care [33,40]. These data pose key new questions for future work: Does parental care facilitate the evolution of mutation rate, and greater standing genetic variation? And if so, does it help or hinder adaptation in a rapidly changing world?

References

1. Cunningham CB. 2020 Functional genomics of parental care of insects. *Hormones and Behavior* **122**, 104756. (doi:10.1016/j.yhbeh.2020.104756)

2. Toth AL, Robinson GE. 2007 Evo-devo and the evolution of social behavior. *Trends in Genetics* **23**, 334–341. (doi:10.1016/j.tig.2007.05.001)

3. Rubenstein DR *et al.* 2019 Coevolution of Genome Architecture and Social Behavior. *Trends in Ecology & Evolution* **34**, 844–855. (doi:10.1016/j.tree.2019.04.011)

4. Kondrashov AS. 2017 *Crumbling Genome: The Impact of Deleterious Mutations on Humans*. Hoboken, NJ, USA: John Wiley & Sons, Inc. (doi:10.1002/9781118952146)

5. Lynch M. 2016 Mutation and Human Exceptionalism: Our Future Genetic Load. *Genetics* **202**, 869–875. (doi:10.1534/genetics.115.180471)

6. Chak STC, Harris SE, Hultgren KM, Duffy JE, Rubenstein DR. 2022 Demographic inference provides insights into the extirpation and ecological dominance of eusocial snapping shrimps. *Journal of Heredity* **113**, 552–562. (doi:10.1093/jhered/esac035)

7. Settepani V, Schou MF, Greve M, Grinsted L, Bechsgaard J, Bilde T. 2017 Evolution of sociality in spiders leads to depleted genomic diversity at both population and species levels. *Mol Ecol* **26**, 4197–4210. (doi:10.1111/mec.14196)

8. Weyna A, Romiguier J. 2021 Relaxation of purifying selection suggests low effective population size in eusocial Hymenoptera and solitary pollinating bees. *Peer Community Journal* **1**, e2. (doi:10.24072/pcjournal.3)

9. Cornwallis CK, Botero CA, Rubenstein DR, Downing PA, West SA, Griffin AS. 2017 Cooperation facilitates the colonization of harsh environments. *Nat Ecol Evol* **1**, 0057. (doi:10.1038/s41559-016-0057)

10. Royle NJ, Smiseth PT, Kölliker M, editors. 2012 *The evolution of parental care*. First edition. Oxford, United Kingdom: Oxford University Press.

11. Snell-Rood EC, Burger M, Hutton Q, Moczek AP. 2016 Effects of parental care on the accumulation and release of cryptic genetic variation: review of mechanisms and a case study of dung beetles. *Evol Ecol* **30**, 251–265. (doi:10.1007/s10682-015-9813-4)

12. Linksvayer TA, Wade MJ. 2009 GENES WITH SOCIAL EFFECTS ARE EXPECTED TO HARBOR MORE SEQUENCE VARIATION WITHIN AND BETWEEN SPECIES. *Evolution* **63**, 1685–1696. (doi:10.1111/j.1558-5646.2009.00670.x)

13. Pilakouta N, Jamieson S, Moorad JA, Smiseth PT. 2015 Parental care buffers against inbreeding depression in burying beetles. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8031–8035. (doi:10.1073/pnas.1500658112)

14. Pascoal S, Mashoodh R, Shimadzu H, Kilner RM. 2022 Parental care allows genetic variation to accumulate by relaxing selection. (doi:10.1101/2022.05.05.490718)

15. Schrader M, Jarrett BJM, Kilner RM. 2018 Parental care and sibling competition independently increase phenotypic variation among burying beetle siblings: BRIEF COMMUNICATION. *Evolution* **72**, 2546–2552. (doi:10.1111/evo.13607)

16. Paaby AB, Rockman MV. 2014 Cryptic genetic variation: evolution's hidden substrate. *Nat Rev Genet* **15**, 247–258. (doi:10.1038/nrg3688)

17. Scott MP. 1998 The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**, 595–618. (doi:10.1146/annurev.ento.43.1.595)

18. Schrader M, Jarrett BJM, Kilner RM. 2015 Using Experimental Evolution to Study Adaptations for Life within the Family. *The American Naturalist* **185**, 610–619. (doi:10.1086/680500)

19. Duarte A, Rebar D, Hallett AC, Jarrett BJM, Kilner RM. 2021 Evolutionary change in the construction of the nursery environment when parents are prevented from caring for their young directly. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2102450118. (doi:10.1073/pnas.2102450118)

20. Rebar D, Bailey NW, Jarrett BJM, Kilner RM. 2020 An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. *Proc Natl Acad Sci USA* **117**, 2544–2550. (doi:10.1073/pnas.1911677117)

21. Schrader M, Jarrett BJM, Rebar D, Kilner RM. 2017 Adaptation to a novel family environment involves both apparent and cryptic phenotypic changes. *Proc. R. Soc. B.* **284**, 20171295. (doi:10.1098/rspb.2017.1295)

22. Jarrett BJM, Evans E, Haynes HB, Leaf MR, Rebar D, Duarte A, Schrader M, Kilner RM. 2018 A sustained change in the supply of parental care causes adaptive evolution of offspring morphology. *Nat Commun* **9**, 3987. (doi:10.1038/s41467-018-06513-6)

23. Cunningham CB *et al.* 2015 The Genome and Methylome of a Beetle with Complex Social Behavior, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Genome Biol Evol* **7**, 3383–3396. (doi:10.1093/gbe/evv194)

24. Li H, Durbin R. 2009 Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760. (doi:10.1093/bioinformatics/btp324)

25. Kofler R, Orozco-terWengel P, De Maio N, Pandey RV, Nolte V, Futschik A, Kosiol C, Schlötterer C. 2011 PoPoolation: A Toolbox for Population Genetic Analysis of Next Generation Sequencing Data from Pooled Individuals. *PLoS ONE* **6**, e15925. (doi:10.1371/journal.pone.0015925)

26. Kofler R, Pandey RV, Schlotterer C. 2011 PoPoolation2: identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics* **27**, 3435–3436. (doi:10.1093/bioinformatics/btr589)

27. Gautier M, Vitalis R, Flori L, Estoup A. 2022 *f* -Statistics estimation and admixture graph construction with Pool-Seq or allele count data using the R package *poolfstat*. *Molecular Ecology Resources* **22**, 1394–1416. (doi:10.1111/1755-0998.13557)

28. Olazcuaga L *et al.* 2020 A Whole-Genome Scan for Association with Invasion Success in the Fruit Fly Drosophila suzukii Using Contrasts of Allele Frequencies Corrected for Population Structure. *Molecular Biology and Evolution* **37**, 2369–2385. (doi:10.1093/molbev/msaa098)

29. R Core Team. 2019 R: A Language and Environment for Statistical Computing.

30. Wickham H *et al.* 2019 Welcome to the Tidyverse. *JOSS* **4**, 1686. (doi:10.21105/joss.01686)

31. Alexa A, Rahnenfuhrer J. In press. topGO: Enrichment Analysis for Gene Ontology.

32. Gotz S *et al.* 2008 High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research* **36**, 3420–3435. (doi:10.1093/nar/gkn176)

33. Baer CF, Miyamoto MM, Denver DR. 2007 Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nat Rev Genet* **8**, 619–631. (doi:10.1038/nrg2158)

34. Mashoodh R, Sarkies P, Westoby J, Kilner R. 2021 Evolved changes in DNA methylation in response to the sustained loss of parental care in the burying beetle. (doi:10.1101/2021.03.25.436923)

35. Rozen DE, Engelmoer DJP, Smiseth PT. 2008 Antimicrobial strategies in burying beetles breeding on carrion. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17890–17895. (doi:10.1073/pnas.0805403105)

36. Wang L, Dankert H, Perona P, Anderson DJ. 2008 A common genetic target for environmental and heritable influences on aggressiveness in Drosophila. *Proceedings of the National Academy of Sciences* **105**, 5657–5663. (doi:10.1073/pnas.0801327105)

37. Flatt T, Tu M-P, Tatar M. 2005 Hormonal pleiotropy and the juvenile hormone regulation ofDrosophila development and life history. *Bioessays* **27**, 999–1010. (doi:10.1002/bies.20290)

38. Baer CF *et al.* 2005 Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5785–5790. (doi:10.1073/pnas.0406056102)

39. Wang Y, McNeil P, Abdulazeez R, Pascual M, Johnston SE, Keightley PD, Obbard DJ. 2022 Variation in mutation, recombination, and transposition rates in *Drosophila melanogaster* and *Drosophila simulans*. (doi:10.1101/2022.09.12.507595)

40. Lynch M, Ackerman MS, Gout J-F, Long H, Sung W, Thomas WK, Foster PL. 2016 Genetic drift, selection and the evolution of the mutation rate. *Nat Rev Genet* **17**, 704–714. (doi:10.1038/nrg.2016.104)

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Author Contributions: RM and RMK conceived of the experiments. RM and AT conducted the experimental work. RM and AM analysed the data. RM and RMK wrote the paper. All authors discussed the results and commented on the manuscript.

Figures & Tables

Table 1. Median theta and pi values for each population evolving under Full Care (FC) and No Care (NC) for each block. Delta is difference between median values between FC and NC computed separately for each block. All *p*-values have been corrected for multiple testing (* indicated $p < 0.001$).

	Evolving Population				
	Block	FC	NC	Δ (FC-NC)	*p*-value
Theta	1	0.00790337	0.00771818	1.85e-04	1.418e-14*
	2	0.00745356	0.00733697	1.17e-04	3.398e-15*
Pi	1	0.00841688	0.00845359	-3.67e-05	0.063
	2	0.00841374	0.00814727	2.66e-04	4.400e-16*

[1]*Non-parametric Kruskall-Wallis test, df=1*

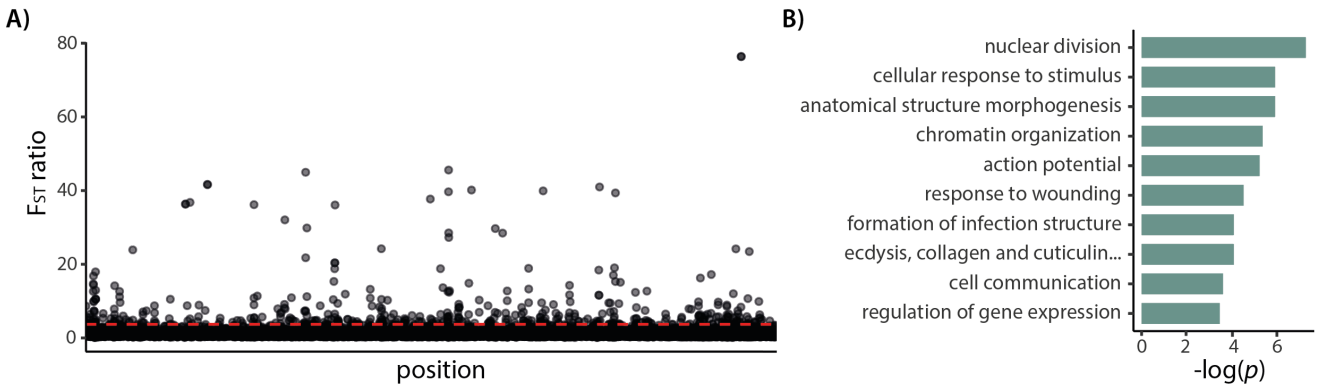
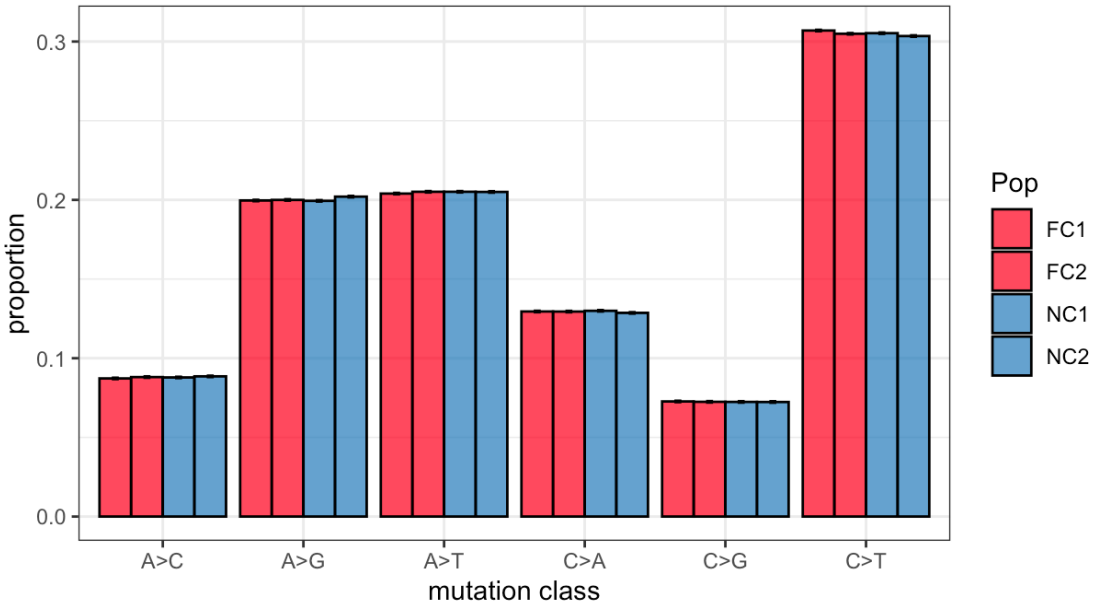


Figure 1. (A) F_{ST} ratios for each 500kb overlapping windows sorte by position. Dashed red line indicates 99th percentile. (B) Top representative enriched GO terms (biological processes) for the most diverged genes between Full Care and No Care populations.

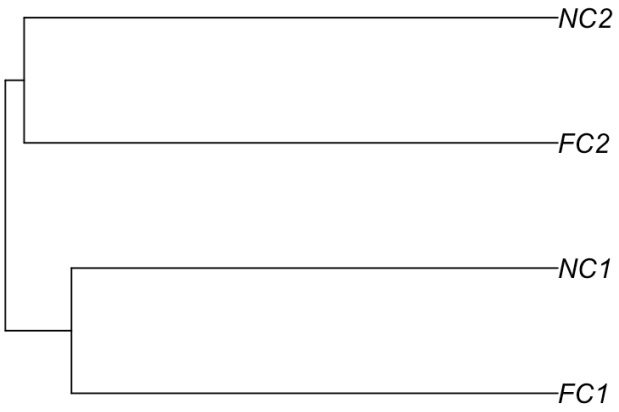
Supplementary Information

Supplementary Table 1. Mapping statistics of pooled libraries for both replicates of populations evolving in the presence of parental care (Full Care; FC1 and FC2) and in the absence of care (No Care, NC1 and NC2).

Population	Total reads (count)	Mapped reads (count)	Mapping rate (%)	Average depth (X)
FC1	140,310,148	122,522,219	87.32	79.86
FC2	163,923,157	143,606,777	87.61	93.34
NC1	163,237,789	140,227,292	85.90	91.55
NC2	151,459,484	131,781,027	87.01	85.11



Supplementary Figure 1. The proportion of each mutation class amongst each population evolving in the presence of parental care (Full Care; FC1 and FC2) and in the absence of care (No Care, NC1 and NC2). Error bars show multinomial 95% confidence interval.



Supplementary Figure 2. Population structure, based on hierarchical clustering of a scaled SNP covariance matrix derived for populations for both replicates of populations evolving in the presence of parental care (Full Care; FC1 and FC2) and in the absence of care (No Care, NC1 and NC2).

Supplementary Table 2. Pairwise genome-wide F_{ST} for populations evolving with (FC1 and FC2) and without parental care (NC1 and NC2) with 95% confidence intervals (CI) of the estimate (SE) and number of SNPs used to calculate F_{ST} (N).

Comparison	F_{ST} estimate	SE (95% CI)	N
FC1;NC1	0.141	4.042E-04	3,462,485
FC1;FC2	0.160	4.192E-04	3,462,485
FC1;NC2	0.173	4.713E-04	3,462,485
NC1;FC2	0.161	4.143E-04	3,462,485
NC1;NC2	0.174	4.674E-04	3,462,485
FC2;NC2	0.170	4.670E-04	3,462,485

Supplementary Table 3. Genes that are adaptive sig.

CSV file

Supplementary Table 4. GO Annotations.

CSV file