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Response to Comment on “Transitions to Asexuality Result in Excess Amino Acid Substitutions”

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Asexual populations experience a reduction in the efficiency of selection when compared with sexual populations. Because asexual lineages of *Daphnia pulex* exhibit no consistent change in mitochondrial base-composition bias, Butlin suggests that this bias is not maintained by selection. On the basis of frequencies of polymorphic directional base changes, we suggest that it predominantly reflects mutation bias.

Base-composition bias is a well-known feature of genomic segments and entire genomes. Unequal frequencies of the four bases may be due to mutation bias, maintained by natural selection, or result from a balance between these two forces (1, 2). If mostly maintained by selection, base-composition bias will change when the efficiency of selection is compromised, with the direction and magnitude of change depending on the relative power and direction of pressure from both mutation and selection.

We recently documented reductions in the efficiency of selection in mitochondrial protein-coding genes of asexual lineages of *Daphnia pulex* (3). Butlin (4) argues that these asexual lineages should exhibit noticeable and consistent change in mitochondrial base-composition bias if the bias was mostly maintained by selection. His analysis revealed no significant difference in the mean proportion of A+T bases among the mitochondrial genomes of 13 sexual and 14 asexual lineages of *D. pulex*. Butlin concluded that selection is not the predominant force determining base-composition bias in the mitochondrial genome of this species. However, we argue that given the recent origin of these asexual lineages, a signature of relaxed selection—even if present—would be difficult to detect using genome-wide estimates of base-composition bias.

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Using parsimony, we estimate that the average genetic distance separating an asexual *D. pulex* from its most recent sexual ancestor equals 23.56 changes across the 13,575 bases of mitochondrial DNA (mtDNA) sequenced. An average of ~90%, or 21.20 changes are transitions, that is, directional changes from A to G or T to C (AT > GC), and from G to A or C to T (GC > AT). Because of their low frequency (~5%), AT > CG and GC > TA transversions can be ignored in our calculations. The mean proportion of A+T bases in the 14 sexual mitochondrial genomes equals 61.5%. Following Butlin's reasoning (4), we assume that selection maintains stationarity of base composition in sexuals and that mutation bias is absent so that the probability of mutation for a given base would be proportional to its frequency in the genome (a rather unrealistic assumption, particularly in mitochondrial DNA). If selection were absent in asexuals, a typical asexual branch would have accumulated an average of 13.04 changes from AT > GC [21.20 changes × 0.615 (frequency of A+T bases)] and 8.16 changes from GC > AT [21.20 changes × 0.385 (frequency of G+C bases)], reducing the mean %A+T from 61.5% [(8348.63 A+T bases/13,575 total bases) × 100] to 61.46% [(8343.75 A+T bases/13,575 total bases) × 100]. Even in the absence of mutational stochasticity, this difference would be statistically difficult to detect given that the standard error around the mean %A+T in the 14 sexual genomes equals 0.027735%.

The reasons for mitochondrial base-composition bias may be better investigated using a method (5) that is analogous to the

McDonald-Kreitman test of neutral molecular evolution. Using this method, we determine the direction of polymorphic base changes and compute the ratio of the numbers of AT > GC to GC > AT transitions along external (E_{AT}/E_{GC}) and internal (I_{AT}/I_{GC}) branches of our phylogeny. The ratios will be close to 1 and equal when base-composition bias is stationary and a result of mutation bias. This is because under stationarity, the frequency of changes from AT > GC must equal that from GC > AT, and under neutrality, patterns of variability observed in the more recent and the more distant past reflect the same mutation process. When using silent base changes, which do not result in a change of the encoded amino acid and are therefore least likely to be affected by selection, we find that estimates for E_{AT}/E_{GC} and I_{AT}/I_{GC} are not significantly different from 1 (sexual branches: $E_{AT}/E_{GC} = 1.012$, $G = 0.003$, $P = 0.956$; $I_{AT}/I_{GC} = 1.138$, $G = 0.257$, $P = 0.612$; asexual branches: $E_{AT}/E_{GC} = 0.942$, $G = 0.059$, $P = 0.807$; $I_{AT}/I_{GC} = 0.727$, $G = 0.236$, $P = 0.627$) and not significantly different from each other (sexual branches: $G = 0.241$, $P = 0.624$; asexual branches: $G = 0.268$, $P = 0.605$). Even when the analysis includes all observed base changes, E_{AT}/E_{GC} and I_{AT}/I_{GC} are not significantly different from 1 (sexual branches: $E_{AT}/E_{GC} = 1.016$, $G = 0.008$, $P = 0.929$; $I_{AT}/I_{GC} = 1.135$, $G = 0.315$, $P = 0.574$; asexual branches: $E_{AT}/E_{GC} = 0.974$, $G = 0.020$, $P = 0.888$; $I_{AT}/I_{GC} = 1.00$, $G = 0.000$, $P = 1.000$) and not significantly different from each other (sexual branches: $G = 0.298$, $P = 0.585$; asexual branches: $G = 0.004$, $P = 0.951$). These results show that base composition has been stationary in both sexuals and their asexual derivatives and suggest that neutral mutation pressure cannot be rejected as the main determinant of base-composition bias in this species.

References and Notes

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