

1 New perspectives on the evolution of within-individual genome 2 variation and germline/soma distinction

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13 14 **Abstract**

15 Genomes can vary significantly even within the same individual. The underlying mechanisms
16 are manifold, ranging from somatic mutation and recombination, development-associated ploidy
17 changes and genetic bottlenecks, over to programmed DNA elimination during germline/soma
18 differentiation. In this perspective piece, we briefly review recent developments in the study of
19 within-individual genome variation in eukaryotes and prokaryotes. We highlight an SMBE 2020
20 virtual symposium entitled “*Within-individual genome variation and germline/soma distinction*”
21 and the present Special Section of the same name in *Genome Biology and Evolution*, together

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58 provided the original work is properly cited.

22 fostering cross-taxon synergies in the field to identify and tackle key open questions in the
23 understanding of within-individual genome variation.

25 **Significance**

26 Genome variation within an individual organism can arise through a plethora of mechanisms.

27 Here we provide a perspective on recent developments in the study of within-individual genome
28 variation as highlighted through a virtual symposium and the present Special Section in *Genome
29 Biology and Evolution*, ranging from polyploidy in bacteria, uniparental genome elimination in
30 fishes, mitochondrial heteroplasmy in molluscs, to germline-restricted chromosomes in insects
31 and songbirds. We outline key open questions that can be addressed through combination of
32 diverse methods and diverse study systems.

34 **Main text**

35 1. Emerging appreciation of diverse forms of within-individual genome variation

36 The dynamic nature of organismal genomes is becoming increasingly appreciated. Perhaps the
37 longest known form of within-individual genome variation is somatic mutation, specifically the
38 movement of transposable elements in maize kernels whose observable phenotype led to the
39 discovery of gene regulation by Barbara McClintock (McClintock 1950, 1956). For the sake of
40 clarity, “germline” refers to the cells or nuclei bearing the genome to be transmitted to the next
41 generation while the term “soma” applies to all other cells that may exhibit genome variation
42 relative to each other, or to the germline. Despite these definitions, we emphasize that some
43 organisms do not necessarily have a clear distinction between the germline and soma, and some

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4 44 forms of within-individual genome variation occur in multicellular and unicellular eukaryotes,
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6 45 and even prokaryotes.
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11 47 Somatic variation may occur through mutations (single-nucleotide changes, small-scale or large-
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13 48 scale structural changes) in individual cells or nuclei during development (Fig. 1A), and is
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15 49 perhaps best studied in the form of complex mutations in human cancer (Chang, et al. 2015;
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17 50 Voronina, et al. 2020), retrotransposition in the human brain (Jönsson, et al. 2020), and single-
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19 51 nucleotide changes in long-lived plants and fungi (Schmid-Siebert, et al. 2017; Hiltunen, et al.
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21 2019; Schoen and Schultz 2019). Another type of somatic variation can arise through somatic
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23 52 recombination, such as in the V(D)J locus of human lymphocytes generating genetic variation
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25 53 for antibodies and T cell receptors (Schatz and Ji 2011). Rather than sequence changes, somatic
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27 54 variation can also arise from ploidy changes during development (Fig. 1B), with prominent
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29 55 examples being the giant polytene chromosomes in the salivary glands of insects (Stormo and
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31 56 Fox 2017) as well as hepatocytes in mammals (Neiman, et al. 2017). Lesser recognized examples
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33 57 are extreme ploidy changes in various groups of unicellular eukaryotes which contain more than
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35 58 one nucleus (Parfrey, et al. 2008) and even some prokaryotes (Angert 2021).
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43 61 Organellar genomes add another dimension to within-individual genome variation in that
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45 62 different genotypes may coexist (heteroplasmy) and segregate differently during development
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47 63 (Fig. 1C) (Stewart and Larsson 2014; Breton, et al. 2015). Mitochondrial heteroplasmy of some
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49 64 bivalves might be particularly prone to such patterns due to their doubly uniparental inheritance,
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51 65 i.e., sex-specific transmission of otherwise coexisting maternal and paternal mitochondria
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4 66 (Zouros, et al. 1994; Capt, et al. 2020; Stewart, et al. 2020), which contrasts sharply with the
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6 67 usually strictly maternal inheritance of animal mitochondria.
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11 69 Uniparental genome elimination, i.e., the elimination of either the maternal or paternal
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13 70 chromosome set during development (Fig. 1D) (Gardner and Ross 2014), may not necessarily
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15 71 lead to within-individual genome variation if elimination only happens during meiosis. However,
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17 72 in some arthropods with paternal genome elimination such as predatory mites, the paternal
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19 73 chromosomes are not silenced but eliminated from the soma (Nelson-Rees, et al. 1980). A form
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21 74 of uniparental genome elimination also exists in some hybrid lineages undergoing
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23 75 hybridogenesis such as *Pelophylax* frogs (Chmielewska et al. 2018), in which a chromosome
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25 76 complement from one parental species is eliminated without recombination during meiosis
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27 77 (reviewed in Lamatsch and Stöck 2009; Dalziel, et al. 2020). Fertilization of the haploid oocytes
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29 78 by one of the parental species regenerates diploidy in offspring, which are thus effectively
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31 79 hemiclinal (Lavanchy and Schwander 2019).
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41 81 An especially peculiar form of within-individual genome variation is caused by programmed
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43 82 DNA elimination during development (Fig. 1E-F). The resulting, often significant,
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45 83 germline/soma genome differences have been observed in a wide range of animals and ciliates,
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47 84 (Wang and Davis 2014; Smith, et al. 2021), two taxa with an early distinction between germline
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49 85 and soma (germline and somatic cells in animals; micronucleus and macronucleus in ciliates). As
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51 86 a detailed review is beyond the scope of this perspective piece, we point the reader to
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53 87 comprehensive reviews of programmed DNA elimination across ciliates (Chalker and Yao 2011;
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55 88 Bracht, et al. 2013; Noto and Mochizuki 2018) and vertebrates (Smith, et al. 2021). During
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4 89 programmed genome rearrangement or chromatin diminution (Fig. 1E), specific regions of
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6 90 chromosomes are eliminated from the differentiating macronucleus in ciliates, as well as from
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9 91 differentiating somatic cells of some nematodes, copepods, and other animals, leading to
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11 92 extensive genome rearrangements in these organisms (Wang and Davis 2014). Recent genomic
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13 93 and transcriptomic data in ciliates, nematodes, and have revealed that eliminated sequences
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15 94 include both germline-expressed genes and repetitive sequences in varying proportions
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17 95 depending on the study system (Wang, et al. 2012; Hamilton, et al. 2016; Wang, et al. 2017).
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22 97 Another form of programmed DNA elimination entails the loss of entire chromosomes during
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24 98 germline/soma differentiation (Fig. 1F), which may either affect sex chromosomes as, for
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27 99 example, in a marsupial species (Close 1984; Wang and Davis 2014) or so-called germline-
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29 100 restricted chromosomes (GRCs) of hagfishes, songbirds, and some arthropods (Wang and Davis
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31 101 2014; Smith, et al. 2021). In lampreys, entire chromosomes are eliminated from somatic cells
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33 102 (Timoshevskiy, et al. 2019) and pioneer transcriptomic studies have revealed that these contain
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35 103 germline-expressed genes and repetitive sequences (Smith et al. 2012; Bryant et al. 2016; Smith
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37 104 et al. 2018). Although some insects have numerous GRCs (Hodson and Ross 2021) and the zebra
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39 105 finch GRC is the largest chromosome of its karyotype (Pigozzi and Solari 1998), genomic and
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41 106 transcriptomic data of any GRCs have been restricted to a 19-kb intergenic region of zebra finch
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43 107 GRCs until recently (Itoh, et al. 2009). It is only recently that a wealth of sequencing data has
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45 108 provided first glimpses into the sequence content of GRCs of songbirds (Biederman, et al. 2018;
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47 109 Kinsella, et al. 2019; Torgasheva, et al. 2019; Pei, et al. 2021) and sciarid flies (Hodson, et al.
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49 110 2021), revealing that GRCs contain many dozens to hundreds of genes, and that they may have
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51 111 existed for millions of years in these lineages (Kinsella, et al. 2019; Hodson, et al. 2021).
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7 113 Taken together, the study of the diverse forms of within-individual genome variation is currently
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9 114 undergoing a transformation towards more diverse study systems across the tree of life.
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12 13 116 2. An SMBE 2020 virtual symposium showcasing diversity of the field

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16 117 Together with *Genome Biology and Evolution* editor-in-chief Laura A. Katz, we had initially
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18 118 planned a symposium to showcase the diversity of the present topic as part of the SMBE 2020
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20 119 meeting, which was to be held in Québec City on June 28 to July 02, 2020, to foster exchange
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22 120 across study systems and career stages. After pandemic events led to a cancellation of the in-
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24 121 person meeting, we organized the symposium as a free-of-charge virtual event on June 29, 2020.
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26 122 The keynote speaker and the six speakers selected from submitted abstracts for the original in-
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28 123 person meeting all agreed to participate in the virtual symposium. We also solicited additional
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30 124 abstracts for virtual poster presentations on short notice, from which we selected six. Nearly 129
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32 125 participants registered, representing 35 nationalities working in 24 countries.
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39 127 The selection of talks and posters spanned the breadth of study systems and career stages among
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41 128 symposium participants. Laurence Hurst gave a 15-minute keynote talk entitled “*The human*
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43 129 *early embryo is a selection arena*”, and 5-minute regular talks from submitted abstracts were
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45 130 given by Esther Angert on “*Challenges faced by highly polyploid bacteria with limits on*
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47 131 *chromosome inheritance*”, Marie-Julie Favé on “*Multi-omics profiles of somatic mutations in*
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49 132 *immune cells from an aging human population*”, Christina Hodson on “*Evolution of a germline*
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51 133 *restricted chromosome in the fungus gnat *Sciara coprophila**”, Mariangela Iannello on “*A*
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53 134 *naturally heteroplasmic clam shows the effects of genetic bottleneck on paternal mtDNA*”,
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4 135 Zuzana Majtanová on “*Chromosome dynamics of sexually-parasitic, unisexual carp gudgeons*
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6 136 (*Hypseleotris*)”, and Jeramiah Smith on “*Programmed genome rearrangement in lamprey*”.
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9 137 Subsequently, the six poster presenters gave 2-minute lightning talks about their posters further
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11 138 highlighting the diversity of study systems, followed by poster presentations in three virtual
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13 139 rooms which allowed participants to move freely between topics and discussions.
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18 141 Peak attendance was around 120 participants and our impression was that the real-time virtual
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20 142 symposium with written chat function, combined with a permanent written discussion board,
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22 143 encouraged participants, and especially early-career researchers, to ask questions in a written
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24 144 manner on both platforms, allowing speakers to respond to questions in spoken and written form
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26 145 as time permitted. Taken together, we believe that the free-of-charge virtual format with shorter
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28 146 talks led to participation of researchers from across the world, at all career stages, and may have
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30 147 ultimately increased diversity in this symposium beyond what would have been possible at an in-
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32 148 person symposium.
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39 150 3. A Special Section with new insights into within-individual genome variation
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41 151 In this Special Section of *Genome Biology and Evolution*, we synthesized some of the key
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43 152 insights discussed at the virtual SMBE symposium. Four of the symposium speakers contribute a
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45 153 manuscript with their respective coauthors, and we believe that this selection of manuscripts
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47 154 highlights the diversity of study systems, methods, and concepts for tackling key questions of the
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49 155 field.
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4 157 Angert (2021) reviews a phenomenon that many eukaryote biologists are probably not aware of –
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6 158 polyploidy in bacteria. Some firmicute bacteria are highly polyploid and produce intracellular
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8 159 offspring instead of binary fission, leading to some chromosome copies effectively having a
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10 160 somatic role by not being passed on to the offspring (Angert 2021).
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16 162 Majtánová et al. (2021) show that hybrid carp gudgeons undergo uniparental genome elimination,
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18 163 effectively resulting in hybridogenesis. The authors also reveal that genome elimination occurs
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20 164 pre-meiotically during the juvenile stage, followed by the duplication of the other chromosome
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22 165 complement before meiosis entry (Majtánová, et al. 2021). This means that diploid somatic cells
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24 166 bear one copy of each parental species genome, whereas pre-meiotic germline cells bear two
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26 167 copies of one parental genome.
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32 169 Iannello et al. (2021) investigate mitochondrial heteroplasmy in a bivalve species with doubly
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34 170 uniparental inheritance. Their results reveal pronounced differences in mitochondrial genotypes
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36 171 among different tissues, possibly as a result of a strong bottleneck early during development
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38 172 (Iannello, et al. 2021).
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43 174 Hodson & Ross (2021) review the diversity of germline-restricted chromosomes in dipteran
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45 175 insects, showcasing the known distribution of GRCs among Sciaridae (dark-winged fungus
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47 176 gnats), Cecidomyiidae (gall gnats), and Chironomidae (non-biting midges). Depending on the
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49 177 taxon, these insects exhibit a single and up to dozens of GRCs with either paternal, maternal, or
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51 178 unbiased inheritance (Hodson and Ross 2021). The authors discuss the potential of genome
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4 179 sequencing for a deeper understanding of GRCs and highlight key questions regarding the
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6 180 evolution of GRCs in dipteran insects.
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11 182 Finally, Asalone et al. (2021) present a transcriptomic-based pipeline that they modified to detect
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13 183 germline-restricted sequences in zebra finch. This method relies on aligning whole genome
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15 184 sequencing reads to a germline genome assembly to detect germline-specific sequences based on
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17 185 read depth. Their approach identifies several newly identified germline-restricted contigs, 51 of
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19 186 which they validated by qPCR.
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25 188 4. Next steps towards elucidating the evolution of within-individual genome variation
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27 189 This Special Section highlights the diversity of within-individual genome variation both in terms
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29 190 of study systems and methods, and that the field is further progressing thanks to the development
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31 191 of cost-efficient or sample-efficient methods for high-throughput data generation. In particular,
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33 192 we anticipate that the continuous improvement of sequencing read length and quality (Sedlazeck,
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35 193 et al. 2018) will further increase the resolution for detecting different types of somatic variation,
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37 194 ranging from single-nucleotide variants to large-scale structural variants. Similarly, the
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39 195 development of ultra-low-input libraries for long-read sequencing (Kingan, et al. 2019) promises
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41 196 the opportunity of studying within-individual genome variation in organisms with small bodies
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43 197 and/or tissues. However, there is a disconnect between signal/noise in sequencing data and actual
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45 198 chromosome structure which may remain for some genomic regions until accurate megabase-
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47 199 scale reads are available (Peona, et al. 2018), and we therefore emphasize the importance of
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49 200 validating complex genomic results with molecular cytogenetic methods (Deakin, et al. 2019).
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4 202 Which forms of within-individual genome variation are stochastic vs. fulfill a biological function
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6 203 remains elusive (Box 1), as well as what biological function that might be. The latter is
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8 204 exemplified by the phenomenon of programmed DNA elimination which has been proposed to
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10 205 either be a means to limit selfish genetic elements to the germline or to minimize antagonistic
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12 206 pleiotropy of genes that are beneficial for the germline but deleterious for the soma (Smith et al.
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14 207 2012; Wang and Davis 2014; Smith 2017). Comparisons of closely related species are necessary
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16 208 to solve such “chicken or egg” problems, as well as developmental and functional genomics of
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18 209 key candidate genes across different developmental stages. To conclude, the time may have
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20 210 come for agnostic “fishing expeditions” to test whether within-individual genome variation,
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22 211 especially in the form of massive germline/soma genome differences, are the odd exception or
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24 212 the overlooked rule across the Tree of Life.
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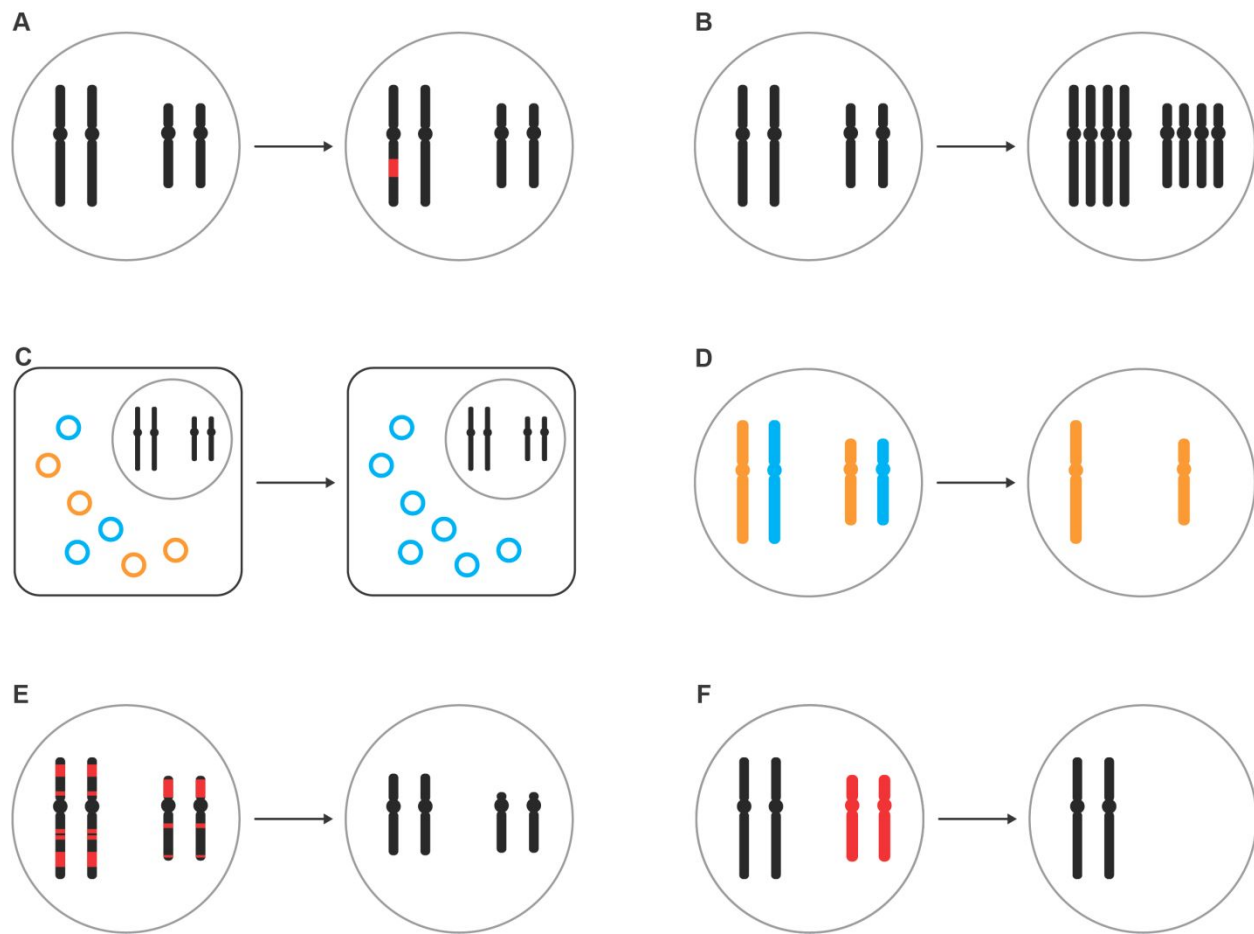
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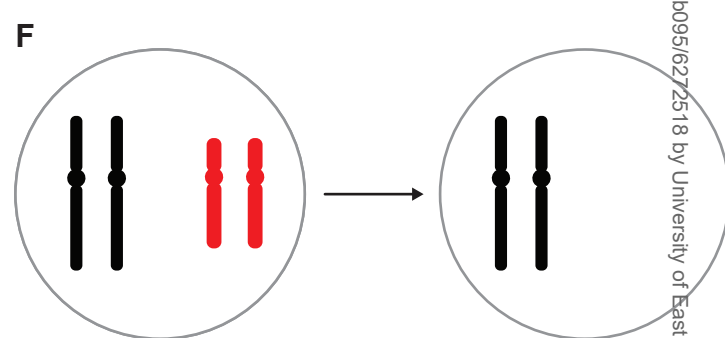
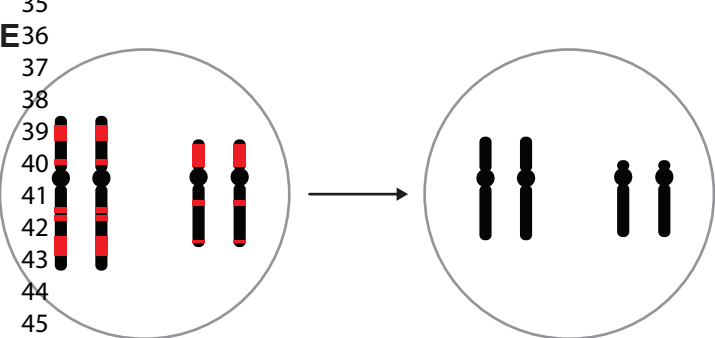
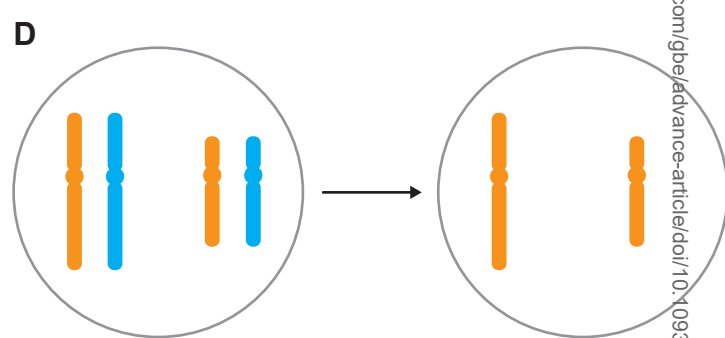
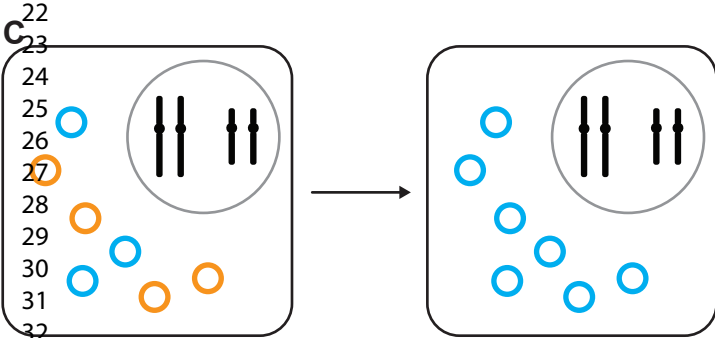
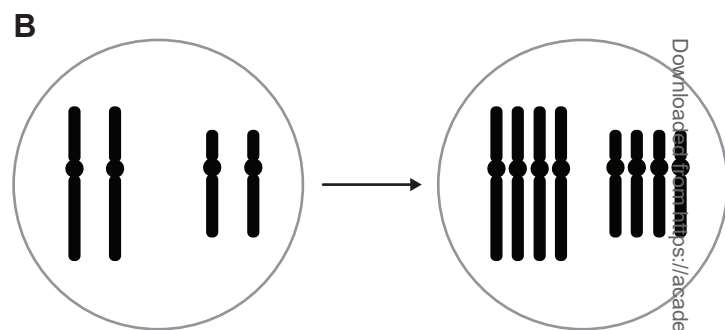
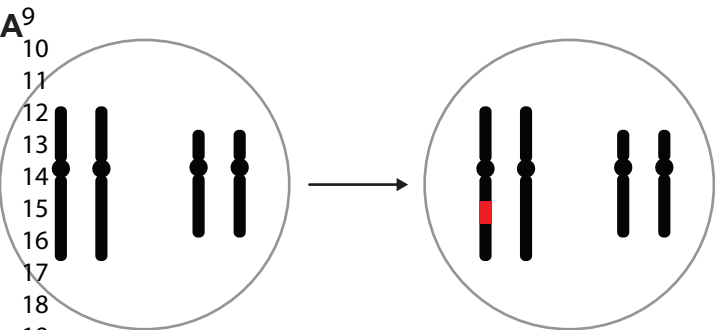
355 **Figure 1: The diversity of within-individual genome variation.** The patterns to the left of each
 356 arrow reflect the individual's genome as inherited from the parental generation and to be
 357 transmitted to the offspring ("germline"), while the patterns to the right of each arrow illustrate
 358 genome variation in some cells or nuclei of the individual ("soma"), although further variation
 359 may exist within germline and soma, respectively. (A) Somatic variation (red) generated by
 360 somatic mutation or somatic recombination. (B) Somatic variation generated by ploidy change.
 361 (C) Within-individual mitochondrial heteroplasmy (orange vs. blue). (D) Uniparental genome
 362 elimination of either maternal or paternal chromosomes (orange vs. blue). (E) Programmed DNA
 363 elimination of chromosome fragments (red; also known as programmed genome rearrangement
 364 or chromatin diminution) from the somatic genome. (F) Programmed DNA elimination of entire
 365 chromosomes (red; e.g., germline-restricted chromosomes) from the somatic genome. Shown are
 366 schematic illustrations of a karyotype with metacentric chromosomes inside a nucleus (grey
 367 circle), though some of these mechanisms may also apply to holocentric chromosomes of
 368 eukaryotes or circular chromosomes of prokaryotes. Note that some of these forms of variation
 369 may also arise during meiosis, leading to within-germline genome variation.

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4 371 **Box 1: Key questions for the study of within-individual genome variation and**
5 372 **germline/soma distinction.**

- 6
7 373 1. How common are the different forms of within-individual genome variation across the tree of
8 374 life?
9 375 2. What are the beneficial, neutral, or deleterious effects of the different forms of within-
10 376 individual genome variation?
11 377 3. Are there currently unknown forms of germline/soma, within-soma, or within-germline
12 378 genome variation that await discovery with new sequencing technologies?
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