Calling structural variants with confidence from short-read data in wild bird populations

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Abstract

Comprehensive characterisation of structural variation in natural populations has only become feasible in the last decade. To investigate the population genomic nature of structural variation (SV), reproducible and high-confidence SV callsets are first required. We created a population-scale reference of the genome-wide landscape of structural variation across 33 Nordic house sparrows (*Passer domesticus*) individuals. To produce a consensus callset across all samples

using short-read data, we compare heuristic-based quality-filtering and visual curation (Samplot/PlotCritic and Samplot-ML) approaches. We demonstrate that curation of SVs is important for reducing putative false positives and that the time invested in this step outweighs the potential costs of analysing short-read discovered SV datasets that include many potential false positives. We find that even a lenient manual curation strategy (e.g. applied by a single curator) can reduce the proportion of putative false positives by up to 80%, thus enriching the proportion of high-confidence variants. Crucially, in applying a lenient manual curation strategy with a single curator, nearly all (>99%) variants rejected as putative false positives were also classified as such by a more stringent curation strategy using three additional curators. Furthermore, variants rejected by manual curation failed to reflect expected population structure from SNPs, whereas variants passing curation did. Combining heuristic-based quality-filtering with rapid manual curation of structural variants in short-read data can therefore become a time- and cost-effective first step for functional and population genomic studies requiring high-confidence SV callsets.

Keywords: structural variation, short-reads, high-confidence variants, rapid manual curation, curation strategies, putative false positives

Significance statement

Calling and genotyping structural variation with short-read re-sequencing data has been facilitated by a broad range of bioinformatic tools, but can be fraught with very high false positive rates. To address this problem, we apply heuristic-based filtering in tandem with rapid manual curation, resulting in significant reduction of putative false positive calls from ~30% to

80% with the most lenient curation strategy, depending on variant class. Given the substantial reduction in putative false positives for downstream callsets even when applying only minimal manual curation effort, we recommend that detection and genotyping of structural variants for population genomic re-sequencing studies should be followed by both heuristic-based quality-filtering and manual curation, a time- and cost-effective step for enriching callsets with high-confidence variants, i.e. putative true positives.

Introduction

Structural variants (SVs; e.g. insertions/deletions, inversions, and duplications) have long been recognized as important in evolutionary processes (Sturtevant 1921; Dobzhansky 1937; Lynch 2007; Noor et al. 2001; Fuller et al. 2018). However, characterising SVs in genomic data has presented an enduring challenge (Bertolotti et al. 2020; Cameron et al. 2019; Mahmoud et al. 2019). Recent technological advancements now make it possible to accurately characterise a broader range of SVs across the genomes of wild organisms. Resulting examples have highlighted the importance of SVs in both evolutionary and conservation-oriented contexts. For example, large-scale inversions play key roles in intraspecific life history polymorphisms in white-throated sparrows (*Zonotrichia albicollis*) (Merritt et al. 2020) and ruffs (*Calidris pugnax*) (Küpper et al. 2016; Lamichhaney et al. 2016), while small SVs (<100 bp) have also been found to play important roles in adaptation and speciation, for example in cichlid fish (Kratochwil et al. 2019; McGee et al. 2020). On the other hand, deleterious SVs may be overrepresented in small populations (Wold et al. 2021). As such, inquiries into the fitness effects of SVs (Gaut et al. 2018; Zhou et al. 2019) and their relative contribution to mutational load are becoming increasingly important for applied conservation genomics (Wold et al. 2023).

Part of the renewed interest in SVs and their identification is driven by advances in bioinformatic tools that facilitate the detection of SVs in sequencing data. However, considerable challenges still remain related to the incidence of false positives, which are known to far exceed the proportion of true positives—even for SVs discovered using short-read data at recommended (>20x) coverage (Belyeu et al. 2021; Wold et al. 2023). For example, in a recent study on structural variation in 492 Atlantic salmon (re-sequenced at an average 8.1x coverage with Illumina short-reads), Bertolotti et al. (2020) reported that up to 91% of identified SVs were false positives after visual inspection with Samplot (Belyeu et al. 2021). Such high false discovery rates have also been reported elsewhere (Cameron et al. 2019; Kosugi et al. 2019) and highlight dangers of relying on bioinformatic approaches alone to identify SVs, particularly when using short-read sequencing data. While long-read sequencing and chromatin-conformation capture for genome assembly and SV detection can help to mitigate this problem (Liao et al. 2023; Mérot et al. 2020; Sirén et al. 2021), using short-read mapping approaches will continue to play a prominent role given the low costs involved, the large amounts of short-read data available and the continued prevalence of study systems possessing only a single reference genome assembly. Furthermore, many studies focusing on wild organisms may be limited in terms of both time and computational allocations; for example, to test or compare outputs from multiple short-read SV discovery tools, which tend to be particularly resource-hungry (Wold et al. 2023). As such, guidelines for time- and cost-effective identification of SVs from short-read data are of particular value, in order to address the current high false positive rates and associated risks of misleading downstream analyses.

To address the issue of high false positive rates, several approaches have been developed, particularly the combined use of multiple tools ("ensemble algorithms") to try to reduce error rates by intersecting variant calls (Ho et al. 2020), and visual inspection ("manual curation") of all identified SVs (Belyeu et al. 2021; Bertolotti et al. 2020). Ensemble approaches can still show high false discovery rates (Cameron et al. 2019; Schikora-Tamarit and Gabaldón 2022), while traditional manual curation methods, in for example the Integrative Genome Viewer (IGV), can be time consuming, though automation of the curation process has the potential to improve the latter approach substantially (Belyeu et al. 2021). In Bertolotti et al. (2020), the bioinformatic approach using LUMPY/smoove (Layer et al. 2014) identified over 165,000 SVs across all 492 individuals. All SVs were visually inspected by a team of curators, taking 5.73 (8-hour) days on average per curator, a reasonable investment given the potential cost incurred by including a substantial proportion of putative false positive calls.

Here, to provide insights into the reliability of short-read sequencing data for SV detection, we use whole genome medium-coverage (~10x) short-read data from Fennoscandian house sparrows (*Passer domesticus*) to examine the structural variation landscape in a species with a relatively compact vertebrate genome size (~1.3Gb; Challis et al. 2023). By visualising different classes of SVs from multiple individuals of the same genotype we improve upon Bertolotti et al.'s automated method using Samplot/PlotCritic. We increase the efficacy and rapidity of manual visual curation by allowing the curator to contrast expected genotypes in a consistent order (2 to 3 individuals of homozygote wildtype, heterozygote, and homozygote alternate for polymorphic variants; or only individuals homozygous for wildtype or homozygote alternate alleles) (**Fig. 1**). Using this improved manual application of Sampot/PlotCritic, we demonstrate that putative false

positive rates are high in short-read data from a wild bird species, and show there is a clear need for visual curation of SVs prior to downstream analyses. We also examine the trade-off between lenient (e.g. using a single curator) versus stringent curation strategies (e.g. using multiple curators) and investigate to what extent these strategies agree in relative proportions of putative false positives rejected and high-confidence variants retained.

Results

Accurate Structural Variant Detection

To ensure accurate SV detection, we built upon the strategies recommended by Bertolotti et al. (2020) using a single generalist program followed by automated manual curation. Rather than adopt an "ensemble algorithm" approach by intersecting calls from multiple programs, which has been shown to result in the retention of a substantial proportion of false positives (Cameron et al. 2019; Mahmoud et al. 2019; Wold et al. 2021), we instead used the generalist structural variant caller LUMPY (Layer et al. 2014) to call larger (>20bp) SVs (deletions, duplications and inversions) from aligned short-reads. We then genotyped the resulting calls with SVTyper (Chiang et al. 2015) and added annotations for fold-change in sequencing depth for SV calls compared to their flanking regions with Duphold, via the smoove pipeline (Pedersen et al. 2020). This produced an initial population-wide VCF of 15,029 deletions, 3,430 duplications and 1,188 inversions (**Table 1**).

As recently recommended by Wold et al. (2023), we then filtered raw deletions and duplications based on call-quality, using the Duphold annotation "DHFFC" ("duphold flank fold-change"). DHFFC is a heuristic metric quantifying the degree of fold-change reasonably expected in regions flanking a putative true positive deletion or duplication; it is therefore not applicable for

inversions) (Pedersen and Quinlan 2019). Duphold (call-quality) filtering rejected 7.6% of raw deletions (filtered for DHFFC < 0.7) and 25.4% of raw duplications (filtered for DHFFC > 1.3; see Methods) as putative true positives for downstream manual curation (**Fig. 2B**, **Table 2**). As recommended by Wold et al. (2023), call-quality filtering with Duphold was followed by genotype-quality filtering of individual genotypes, based on Mean Smoove Heterozygote Quality annotations (MSHQ). MSHQ (genotype-quality) filtering rejected 9% of raw deletions, 41% of duplications and 8% of (1,094) inversions.

Evaluating Alternative Strategies for Rapid Manual SV Curation

To build upon the recommendations of Bertolotti et al. (2020) for manual curation speed and efficiency, we evaluated curation performance of SVs using both deep-learning and single versus multiple human curators (**Fig. 2A**). We first applied Samplot-ML on the full deletion callset, a pipeline for automated curation by deep-learning, currently only available for deletions (Belyeu et al. 2021). This step rejected ~5% of all raw deletions, supporting earlier insights from Belyeu et al. (2021) that Samplot-ML removes similar proportions of deletions as Duphold (call-quality) filtering (**Fig. 2B, Table 1, Table 2**).

To evaluate the speed and efficiency of removing putative false positives through automated manual curation, we compared curation performance between single and multiple curators by applying the curation approach demonstrated and validated in Bertolotti et al. (2020) and Belyeu et al. (2021). To ease manual curation, we chose to only examine SVs represented by a minimum of 3 individuals per genotype class (i.e. three homozygote reference, three homozygote alternate, three heterozygote; hereafter referred to as the "genotype-frequency filtered" subset) using

Samplot and PlotCritic (formerly referred to as SV-Plaudit; Belyeu et al. 2018, 2021). Filtering by this genotype-frequency threshold removed 77% of all raw deletions (11,568 removed), 62.7% of all raw duplications (2,149 removed), and 78.8% of all raw inversions (936 removed) (**Table S2)**; retaining a total of 3,461 deletions, 1,281 duplications and 252 inversions for manual curation. We then randomly sampled (with replacement) and plotted two to three individuals of the resulting genotype class for each resulting SV in Samplot (see **Fig. 1** for an example of the Samplot layout used in this study; **Fig. S3** for a putative true positive deletion; **Fig. S4** for a putative false positive inversion; further examples in guidelines for identifying SVs in Supplementary Materials), using the PlotCritic interface to record curators' alternative answers to the question: "Is this a real SV?": "Yes", "Maybe" or "No" (see **Fig. S2** for an example screenshot of the PlotCritic interface). The "Maybe" category allowed for more rapid curation by reducing time evaluating more ambiguous borderline cases, while allowing curators to focus on primarily removing obvious putative false positives. In this case we chose to consider calls scored as either "Yes" or "Maybe" as putative true positive calls in downstream analyses, but a more stringent callset could be easily created by extracting only "Yes" scores from PlotCritic reports. Each separate curator independently examined the "genotype-frequency filtered" subset, comprising a total of 4,994 structural variant images (3,461 deletions, 1,281 duplications, and 252 inversions), spending an average of 3 to 5 seconds per image, amounting to only ~4.2 to 6.9 hours of total curation time per person.

Variation in the total number of rejected putative false positive SVs was observed between curators and is helpful to inform future standardisation of curation strategies. Firstly, this variation probably reflects differences in curation approach between curators, despite similar

search images for putative true positive (high-confidence) SVs (see guidelines used to train curators for identifying SVs in Supplementary Materials). This may occur where a lenient curation strategy is defined as focusing on the removal of obvious putative false positives from a callset, rather than attempting to unambiguously identify true positive calls while requiring that all individuals in a given Samplot showed correct genotypes. Only three curators (G.D., H.B., E.G.) used the "Maybe" category, while the most stringent curator (A.B.) did not (only answering "Yes" or "No"), allowing for comparison of individual variation in curation stringency. This further restricted the final callset of the most stringent curator, because "Yes" and "Maybe" calls were all merged and considered downstream as putative true positive (high-confidence) SVs. For callsets curated by the most lenient curator (G.D.), the putative false positive rate was highest for duplications (78% rejected), but substantially lower for deletions (29% rejected) and inversions (30% rejected; **Table 2 and Table S3**). In contrast, of the variants retained as putative true positive (high-confidence) variants after the intersection of all four curator callsets, the putative false positive rate was much higher for both duplications (97% rejected) and inversions (95% rejected), compared to deletions (64% rejected) (**Table 2 and Table S3**). Variants retained by the most stringent curator (A.B.) were largely a subset of the high-confidence variants retained by all the other curators. Most importantly, >99% of variants rejected as putative false positives by a single, lenient curator (2073) were also rejected by all other three independent curators (2065). This demonstrates near-complete agreement between the most lenient and stringent curation strategies in terms of rejection of obvious putative false positive SV calls. In addition, putative false positive deletions rejected by both a single curator applying a lenient strategy and all four curators (stringent strategy) do not appear to show

significant population structure (**Fig. 3F**; **Fig. S15, Table S3E**) compared to curated deletions (**Fig. 3A**, **Table S3A, Fig. S8**).

High-confidence variants passing manual curation were largely subsets of variants retained by call-quality (Duphold) and genotype-quality (MSHQ) filtering alone: all but two variants retained by the intersection of all four curator callsets (the most stringent callset) passed both call/genotype-quality filtering: 1242/1243 deletions, 36/37 duplications, and all inversions (13/13). In this sense, genotype-frequency filtering, stringent curation and/or the addition of successive curators essentially performs as both heuristic call/genotype-quality filtering alone, though possibly with substantially fewer putative true positive (high-confidence) SVs retained than with a single, lenient curator. For example, all but one deletion retained by the most lenient curator (G.D.) as putative true positives (2,456/2,457) were also retained by both Duphold-filtering and Samplot-ML, while 91% of duplications (262/287) were kept by Duphold-filtering. Additional genotype-quality filtering with MSHQ on variants retained by the most lenient curator (G.D.) kept 99.9% (2,454/2,457) of deletions, 81% (231/287) of duplications, and 98% (174/177) of inversions. That ~20% of all duplications retained by the single, lenient curator were rejected by call/genotype-quality filtering may be attributed to the fact that G.D.'s curation strategy prioritised the removal of obvious putative false positive SVs (in contrast to the most stringent curation strategy), while also allowing for occasional individual genotypes to be incorrect for a given Samplot image (in contrast to genotype-quality filtering). Given that the average coverage for short-read data used in this study was ~10x, it may be reasonable to assume that duplications would be especially prone to higher genotyping errors. This could lead to the rejection of potential putative true positive (high-confidence) variants by genotype-quality filtering (e.g. with MSHQ) alone, in low-to-medium (<20x) coverage data.

Creation of a "genotyped-frequency filtered" subset of variants selected for curation (whereby only common SVs represented by at least 3 individuals per genotype class were considered for curation) essentially functioned as a form of indirect filtering for minor allele frequency and size. In removing rarer variants by selecting only common SVs for curation, the proportion of larger (>500bp) was also substantially reduced, without the application of hard size cut-offs (**Table S2**). As expected, larger variants called by smoove thus do appear to rarer than our genotype-frequency threshold. However, not all larger variants were removed by genotype-frequency filtering: while most (>90%) but not all deletions and inversions larger than 500 Kb were removed, only ~65% of duplications larger than 500 Kb were removed (**Table S2**). Therefore, stringent filtering by genotype-frequency did not substantially change the maximum size classes of variants to be curated (**Table S2**), relative to raw callsets.

Manual curation further reduced the number and relative proportions of retained SVs of different size classes, relative to raw (uncurated), Samplot-ML-filtered and Duphold-filtered (call-quality filtered) callsets (**Table 1, Table 2, Table S2**), though less markedly when applying only the most lenient curator (G.D.) (**Table 1, Table 2, Fig. 2, Fig. 4**). More than twice the total number of putative true positive (high-confidence) SVs were retained by a single, lenient curator (2,921 SVs retained) relative to all four curators (1,293 SVs retained) (**Table 1, Table S3**). Putative true positive inversions and duplications retained by a single curator were nearly 10 times more numerous than those retained by all four curators, when contrasting the most lenient strategy (single, lenient curator, using both "Yes" and "Maybe" scores) versus the most stringent strategy (only calls retained after intersection between all four curators, including the strictest curator

whom only used "Yes" scores). Following curation by all four curators, we observed a marked reduction in the reported maximum length of variants for each SV class as well as a reduction in the median length for deletions and duplications. Both the maximum and median lengths of retained inversions and duplications were markedly higher for a single curator than for all four curators, but not for deletions. The size distributions of retained deletions were similar between single and multiple curators (**Table 2, Fig. S11**), though almost double the number of larger deletions (from 1 Kb to 5 Kb) were retained by a single curator (**Fig. S12**). However, no duplications or inversions >1 Kb were retained by all four curators, while a single curator retained 76 duplications (from 1,011 bp to 2.1 Mb) and inversions (1,406 bp and 2,057 bp) >500 bp in length (**Table 2, Fig. S3 and S14**). Only four putatively true positive SVs exceeded 10 Kb in size, all of which were duplications identified by a single curator, ranging from ~1 Kb to 2.1 Mb. In contrast, the maximum size of duplications retained by all four curators was limited to <500bp (**Table 1, Table 2**). We therefore suggest that while one or two curators may be capable of discarding the bulk of obvious putative false positives, adding subsequent curators may increase the putative false negative rate. We cannot conclude this definitively, as we have not orthogonally verified that the Samplot-rejected SVs were indeed false positives, nor have we orthogonally validated curated SVs. However, because several previous studies have validated Samplot images representing putative true positive variants using e.g. ddPCR or long-read sequencing (Belyeu et al. 2021; Bertolotti et al. 2020), manual curation in Samplot/PlotCritic has in itself been considered an independent validation method to estimate false discovery rates, even without a truth callset (Wold et al. 2023; Belyeu et al. 2021). We therefore refer to both lenient (single curator) and stringent (multiple curator) curated callsets as "high-confidence" SV callsets, *sensu* Bertolotti et al. (2020).

Population structure is captured by curated SVs, but not by rejected SVs

As further supporting evidence that high-confidence structural variant callsets included substantially fewer putative false positives, we compared population structure between curated SVs, Duphold-rejected SVs, curator-rejected SVs, all SNPs and short indels as well as downsampled SNPs (to same number as SVs). Assuming that large SVs and SNPs are largely governed by the same evolutionary forces of genetic drift, mutation, recombination and selection (Lynch 2007; Sjödin and Jakobsson 2012), we hypothesised that even relatively low numbers of common, high-confidence SVs should capture similar patterns of population structure as SNPs and short indels, while SVs rejected as putative false positives would not. In line with our expectations, high-confidence deletions retained by all four curators (**Fig. 3A; Fig. S8**) best captured population structure (**Fig. 3C**) inferred from ~30 million SNPs (**Fig. 3B**) and 600,575 short indels (**Fig. S10A**) compared to downsampled SNPs (**Fig. 3D, Fig. S10B**). In contrast, Duphold-rejected deletions (**Fig. 3E**) and curator-rejected deletions (**Fig. 3F, Fig. S15A**), duplications (**Fig. S15B**) and inversions (**Fig. S15C**) all largely failed to recover expected patterns. Similar to Bertolotti et al. (2020), we also found that high-confidence deletions best recaptured known population structure from SNPs and short indels compared to duplications and inversions, possibly due to the much lower number of variants remaining after curation (**Fig. S8, Table 1**).

To further investigate the potential effect of filtering and manual curation on population structure, we calculated pairwise weighted F_{ST} **(Table S3)** between two individuals for each of four major population clusters ("Trøndelag"; "Pasvik"; "Finland"; "Leka/Vega") for the "high-

confidence" SV callset retained by all four curators together. We found that relative F_{ST} differentiation closely mirrored the relative distance between the major clusters previously identified in the different principal component analyses (PCA) shown in **Fig. 3.** Of all SNP and curated SV or rejected SV callsets shown in **Fig. 3**, the highest weighted F_{ST} values were for all pairwise population comparisons for the curated deletion (1,243) callset (**Fig. 3A),** with strongest differentiation between "Pasvik" and "Leka/Vega" (mean F_{ST} = 0.159 ; weighted F_{ST} = 0.275; **Table S3A**) and weakest differentiation between "Trøndelag" and "Finland" (mean F_{ST} = 0; weighted F_{ST} = 0.040, **Table S3A)**. Similar to PCA analyses, the callset for Duphold-rejected deletions (1,135) failed to recover any pattern of differentiation (**Table S3C**).

Annotation of high-confidence SVs in *Passer domesticus*

To examine the potential impact of SVs, we intersected the high-confidence SV callset with the published annotation for the reference assembly (NCBI accession GCA_001700915.1). High-confidence inversions at least partially overlapped 406 genes, while high-confidence deletions and duplications overlapped 1,277 and 2,570 genes, respectively. As the largest linkage groups, chromosomes 1-5, 1A and the Z chromosome exhibited the highest numbers of SVs, with the exception that no inversions were retained on the Z chromosome, following curation by a single curator (2,921 SVs, **Fig. S5**). Notably, five high-confidence duplications (all 20 Kb to 2.1 Mb in size) were identified on chromosome 20 (**Fig. S19**), following both Duphold filtering and manual visual curation. Fifty annotated genes were found to be completely overlapped by a single 1.4 Mb duplication (position 8119985 to 9530772). All 50 overlapped genes were located with positions 8.16 Mb to 9.46 Mb, ~841 Kb upstream of the *Fm* region, a complex segmental duplication identified on chromosome 20 in the domestic chicken (*Gallus gallus*) genome

(Dharmayanthi et al. 2017; Dorshorst et al. 2011). This potential true positive duplication was detected (as heterozygote or homozygote alternate allele) in over 5% of individuals and passed both Duphold (call-quality) filtering and a lenient curation strategy. However, it was rejected by multiple manual curators (stringent strategy), highlighting the potential trade-off between lenient versus stringent curation strategies (**Fig. S19, Fig. 4)**. Given that all short-read SV callsets should be considered preliminary (Wold et al. 2021), further validation with long-read and/or molecular data will be necessary to confirm putative true positive SVs.

We further annotated the 2,921 large, high-confidence structural variants retained by a single curator with SnpEff (Cingolani et al. 2012). Of 2,457 deletions, 2% were predicted to be of high impact and 98% were modifiers, while 77%, 9%, and 6% were located within 5 Kb of a protein coding gene in intergenic, intronic and upstream regions, respectively. Of 177 inversions, 1% were predicted to be of high impact and 99% were modifiers, while 75%, 13%, 6% and 5% were located within 5 Kb of a protein coding gene in intergenic, intronic, upstream and downstream regions, respectively. In contrast, of 287 duplications, 5% were predicted to be of high impact, 38% of moderate impact and 41% were modifiers, while 41%, 6%, and 29% were located within 5 Kb of a protein coding gene in intergenic, intronic and transcript regions, respectively. High-confidence SV-callsets were also intersected with a newly generated transposable element library for *Passer domesticus* (**Table S6**), using BEDtools v2.29.2 (Quinlan and Hall 2010). High-confidence deletions, duplications and inversions were all found to overlap mostly with LINE/CR1 and LTR transposable elements identified in the repeat library (see **Table S4**). Duplications at least partially overlapped with more transposable elements (1,253) compared to inversions and deletions.

Discussion

The number of population genomic studies on structural variants is increasing rapidly. Our aim here was to contribute to best practice in discovering high-confidence structural variants, using geographically separated populations of house sparrows in Fennoscandia. In order to do so, we built upon recent insights promoting the use of heuristic-based call/genotype-quality filtering (e.g. Liu et al. 2021; Wold et al. 2023) by applying an improved approach for rapid manual curation with Samplot/PlotCritic (Belyeu et al. 2021; Bertolotti et al. 2020). When considering retained high-confidence SVs, we found that these capture similar patterns of population structure to those observed using SNP data. In contrast, SVs rejected by both Duphold-filtering (call-quality filtering) and manual curation failed to recapture expected population structure (**Fig. 3**) and differentiation (**Table S3**) determined with both genome-wide SNPs and curated structural variant callsets, supporting the conclusion that these rejected variants are indeed likely false positives.

Overall, we note that call/genotype-quality filtering alone does not suffice to remove putative false positives. We build upon Wold et al.'s (2023) insights to recommend a time- and cost-effective approach, especially amenable to population genomic and functional genomic projects with resource constraints, e.g. limited to a single reference genome and low-to-medium (<20x) short-read re-sequencing data. In line with earlier studies (Bertolotti et al. 2020; Cameron et al. 2019; Kosugi et al. 2019; Mahmoud et al. 2019) we found high putative false discovery rates of inferred SVs from short-read data, even when applying a single curator and a lenient manual curation strategy. Our results indicate that future studies could benefit in performing

curation/visual inspection of short-read-discovered SVs prior to downstream analyses (Bertolotti et al. 2020) and we recommend different curation strategies based on study objectives below.

SV detection in short-read data

Previous population genomic studies reporting SV callsets from short-read data alone have either applied a single program (Liu et al. 2021; Catanach et al. 2019) or a combination of programs ("ensemble algorithms", Ho et al. 2020; Weissensteiner et al. 2020), but without heuristic-based filtering (but see Liu et al. 2021; Wold et al. 2023; Lee et al. 2023) and/or manual curation. Where relying on short-read data only, it may ultimately be preferable to apply a single algorithm which uses multiple signals to detect SV presence (e.g. read-depth, split-reads and read-pairs combined) coupled with quality-filtering and manual curation, rather than simply overlapping calls from multiple algorithms (Wold et al. 2021, Wold et al. 2023). For example, Cameron et al. (2019) found that no ensemble algorithm (e.g. Parliament2 or SVtools) consistently outperformed individual callers (e.g. LUMPY, Manta, Delly). In a recent comparison of SV caller and genotyper performance on short-read data generated for an endangered parrot, Wold et al. (2023) found that among competing individual callers/genotypers, smoove (LUMPY/SVTyper) retained the largest number of high-confidence SVs following filtering for quality and size. Additionally, the authors recommended smoove among the best choice of short-read SV discovery tools, should computational and financial resources be limited-- as may be the case for many smaller conservation-oriented projects. However, individual callers/genotypers such as smoove are also known to produce high false positive rates (even following filtering by quality, and size), prompting the creation of rapid visual curation methods (Belyeu et al. 2021).

Our most lenient manual curation strategy rejected 29% of deletions, 77.6% of duplications and 30% of inversions (**Table 2**), following filtering by genotype-frequency (see Methods; **Table S2**). Thus, a large fraction of SV calls filtered by genotype-frequency are still likely putative false positives in our study, assuming manual curation with Samplot/PlotCritic is accurate; as supported by prior validation efforts of Samplot images (Belyeu et al. 2021; Bertolotti et al. 2020). Similar manual curation results have been observed in other studies utilising short-read sequencing data. For example, Bertolotti et al. (2020) reported an overall false discovery rate of 91% for SVs initially called with smoove in Atlantic salmon re-sequenced to 8.1x coverage. This study also validated a subset of SVs retained after visual curation in Samplot/PlotCritic with long-read sequencing and found a putative true positive rate of 88% for presence/absence and 81% for genotype across all SV classes. This suggests that the application of a manual curation pipeline can dramatically reduce putative false positive calls.

Considerations and recommendations for rapid manual curation

Though filtering by call- and genotype-quality is a good first step for rejecting likely real false positives (e.g. call-quality filtering with Duphold: **Fig. 3E**; Wold et al. 2023), it currently does not suffice in removing the bulk of obvious false positives (**Table 1, Table 2, Fig. 4;** Belyeu et al. 2021). In turn, automated curation with Samplot-ML identified even fewer variants as putative false positives than call-quality filtering with Duphold, suggesting further fine-tuning on non-human callsets is needed. Therefore, manual curation of short-read-discovered callsets is of particular utility in addressing the current constraints for generating high-confidence SV callsets from short-read data alone.

When performing SV-discovery from short-read data aligned to a single reference, our results indicate that some degree of curation is better than none at all. Importantly, almost all (>99%) of SVs rejected as putative false positives by a single curator were also rejected by all four curators, showing that there is near-complete agreement between curators in the identification of the most obvious putative false positives. These rejected putative false positives (**Fig. 3F, Table S4E**) failed to capture the same degree of population structure as curated deletions (**Fig. 3A, Table S4A**) or SNPs (**Fig. 3B**, **3D, Table 4D**). In addition, a single, lenient curator rejected up to 80% of SVs as putative false positives, demonstrating that that even a minimal investment in time and effort can aid to reject the most obvious putative false positive calls and substantially improve callsets for downstream analyses and validation. Therefore, if the goal of a given study were to remove as many obvious putative false positive SVs while minimising the incidence of putative false negatives, SVs retained by a single curator following SV call/genotype-quality filtering could suffice to be considered as a "high-confidence" callset (*sensu* Bertolotti et al. 2020).

We found that there may be a trend of "diminishing returns" when adding more than two curators, due to an increasing disagreement between what constitutes a putative false positive SV, especially when study objectives between curators may differ (e.g. rejecting obvious putative false positives versus retaining only obvious putative true positives) (**Fig. 2 and Fig. 4)**. Following the addition of a third or fourth curator (the most stringent curators), the percent agreement as to what constitutes a putative false positive decreases substantially between curators (**Fig. 4**, **Table S3**), especially for duplications and inversions. For example, the two most lenient curators agreed on 96% duplications to be rejected (i.e. the remaining 4% had been retained as a putative true positive by one but not both of the curators) (**Fig. 4, Table S3**). In

contrast, the three most lenient curators only agreed that 79% of the duplications rejected by the fourth and most stringent curator were putative false positives. Within this study, the first author (G.D.) was identified as the most lenient curator because they rejected the fewest variants as putative false positives, while the strictest curator (A.B.) rejected the most variants. Therefore, callsets retained by more stringent curation strategies necessarily restricted the total number of retained SVs, when different curator callsets are intersected in order of increasing stringency (as in this study) (**Fig. 4**). In the absence of G.D., the number of SVs retained by our next most-lenient curator or by the most stringent curator alone would have been significantly fewer. At the same time, variants rejected as putative false positives by the most stringent curation strategy but retained by the most lenient strategy are not necessarily all real false positives.

Even with established guidelines for training new curators (see Supplementary Materials), we still observed substantial variation in curation stringency. These differences in curation stringency may in large part reflect subtly different perceived project goals: while the most lenient curator aimed to remove obvious false positives, the most stringent curator retained only the most obvious true positives (i.e. only those conforming strongly to expected search images; see Supplemental Materials), which resulted in large variation in the final callsets retained by different curators. While it is likely that individual variation between curator stringency cannot be completely avoided, prospective studies could substantially reduce this variation by clearly defining curation goals prior to beginning curation. To achieve consistency, it may be helpful for prospective curators to first agree upon project goals: whether the goal is simply to remove obvious putative false positives (lenient strategy) or to attempt to unambiguously identify putative true positives (stringent strategy), while only allowing for Samplot images represented

by individuals with correct genotypes—though this latter requirement may be unrealistic for SV genotypes determined from low to medium coverage (< 20x) short-read data (Wold et al. 2023). It may also be helpful to practice on model datasets or a subset of the real dataset and then discuss with curators whether any differences in curator stringency may be due to differences in perceived project goals.

Depending on study objectives, the trade-off in sensitivity between stringent and lenient curation strategies can be weighed. For example, lenient curation strategies with one or two curators may help to reduce the risk of discarding less obvious putative true positives, particularly for rarer variants such as large, complex SVs exceeding several kilobases where visual curation is more difficult. In contrast, for functional genomic studies (reviewed in Gudmunds et al. 2022) it may be advisable to take a more stringent approach using multiple curators followed by molecular confirmation before further time-intensive functional work. Regardless of study objectives, we consider the combination of heuristic-based quality-filtering recommendations (Wold et al. 2023) with rapid visual curation of SV callsets an important and easy first step before drawing inferences from population genomic analyses on small (mostly >25 bp to 1 Kb) structural variants in both short-read and long-read genomic datasets.

Furthermore, our results suggest that a stringent curation strategy by multiple manual curators may lead to increased false negative rates, if taking the intersection of calls between all curators (**Fig. 2, Fig. 4**). We identified a minimum of 29% of structural variants designated as putative false positives by visual curation with a single curator and at least 64% with multiple curators. In contrast, quality-filtering removed only ~5% putative false positives and deep-learning removed

only 2% putative false positives from raw calls. A single curator thus offers a considerable improvement in accuracy compared with filtering and automated approaches, and may suffice, at least if the main objective of visual curation is to discard obvious putative false positive SV calls. In contrast, the use of multiple curators greatly increases accuracy, but at the potential cost of reduced sensitivity (**Fig. 2, Fig. 4**).

Notably for deletions however, the number, size range and population structure recovered in principal component analyses all showed remarkable consistency between a single curator and all four curators (**Table 1 and Table 2; Fig. 3 , Fig. 4, Fig. S8**). In contrast, much more variation was identified between single and multiple curators for the number and size range of inversions and duplications (**Table 1 and Table 2; Fig. 4**). A total of 434 structural variants were found to at least partially overlap annotated genes and also mostly conformed (**Fig. S19**) to the relative proportions of unannotated SVs retained by a single lenient curator (**Fig. S5**), while 2,869 short indels partially overlapping genes did not (**Fig. S6**). None of the 50 genes overlapped by duplications (**Fig. S19**) were retained as putative true positives by all four curators (37 duplications), despite both single curator and multiple curator callsets retaining a similar size range of duplications on chromosome 20, from ~95 bp to ~140 Kb. While the size distribution of retained deletions were also largely similar between single and multiple curators (**Table 1, Table 2, Fig. S11)**, only four structural variants retained as putative true positives by multiple curators exceeded 10,000 bp in size, all of which were duplications identified by a single curator. Therefore, adding too many curators will also likely reduce the maximum size of SVs retained as putative true positives, if the intersection of all curation scores is used as in Bertolotti et al. (2020).

We found that filtering by genotype-frequency prior to manual curation substantially (but not completely) reduces the proportion of larger variants further (**Table S2**), implying that larger SVs called with short-read callers (e.g. smoove) are indeed rare (**Fig. S19**). Our lower-than-recommended (Wold et al. 2023) sequencing coverage (~10x) also likely contributes to the relative paucity of larger variants retained, which (to a certain extent) can be detected by a smoove/Samplot approach (Belyeu et al. 2020). In contrast, in directly applying the same filtering and rapid curation approach (as described here) on higher-coverage (~30x) short-read data, Smeds et al. (2024) succeeded in detecting and retaining larger high-confidence deletions, duplications, and inversions exceeding 100 Kb in size—despite overall putative false positive rates similar to this study. Regardless, comprehensive resolution of large and complex SVs will require multiple sequencing technologies (high-accuracy long-reads and short-reads) as well as novel bioinformatic approaches such as pangenome graphs (Sirén et al. 2021). In the absence of these approaches, careful curation of SV calls based on read-mapping-based programs alone can aid in narrowing down the list of putative true positive SVs which may be of biological interest.

To further aid rapid manual curation, we recommend first filtering by genotype-frequency, in order to select and plot at least one to three individuals of each genotype. While this restricts SV discovery to only very common variants, our plotting approach is fully scalable to hundreds of samples (Bertolotti et al. 2020), which would increase the potential for detecting relatively rarer variants represented by 1 to 3 individuals of each genotype. In testing our approach, we did not find any significant disadvantage to SV curation when reducing the number of individuals of each genotype plotted. Rather, the key benefit appears to be able to contrast individuals of the

three genotype classes (homozygote reference, heterozygote, homozygote alternate) in a consistent order, regardless of whether 1 or more individuals are visualised per genotype. Recently, this curation approach using only 2 individuals per genotype class was successfully applied to a study of structural variation across 212 Scandinavian wolves (Smeds et al. 2024), allowing for identification of high-confidence SVs at lower allele frequencies (MAF ≥ 0.01). Crucially, in performing manual curation with Samplot/PlotCritic, Smeds et al. (2024) were able to remove batch effects discernible in the raw SV calls and rejected calls also failed to recapture expected population structure (as found in this study).

We have here defined a putative false positive variant as an SV-call not passing call/genotype-quality filtering or manual curation. Supporting this assumption, rejected variants failed to recapture expected population structure (**Fig. 3D, Fig. 3E, Table S4C, Table S4E).** We note again however, that we have not applied orthogonal evidence to verify that calls identified as putative false positives are not e.g. true positive calls with relatively "poor" concordant/discordant read-pair and split-read signals in Samplot. In theory, it may be possible that even a relatively unambiguous putative false positive (e.g. rejected unanimously by all four curators) could indicate the presence of a true complex variant, which is difficult to resolve with short-read data. However, in line with previous studies (Belyeu et al. 2021; Bertolotti et al. 2020) we suspect that variants identified as false positives in Samplot harbour a disproportionately higher probability of being erroneous, especially when discovered by aligning short-reads to older (e.g. Illumina) reference genomes. Other than high error-rates previously documented for SV discovery tools and short-read data themselves (e.g. due to alignment artifacts), possible sources for erroneous calls could include gaps or mis-assemblies in the reference genome, library

preparation, PCR artifacts or somatic SVs (Mahmoud et al. 2019, Cameron et al. 2019). However, even if putative false positives were to indirectly point towards the presence of a large/complex SV, the number, size and orientation of the smaller calls would likely still be erroneous. If numerous, these erroneous calls could substantially inflate allele counts and downstream population genetic summary statistics (see below). We therefore distinguish here between indirect evidence (e.g. through erroneous mapping signals) for the possible presence of a novel and complex SV, versus direct characterisation of the number of (mostly smaller) putative true positive SVs of specific size and orientation reflecting actual biological differences in genomic architecture across individuals.

Ultimately, it may be impossible to completely avoid the trade-off between removing putative false positives at the cost of removing putative true positives with manual visual curation alone. This trade-off has however nearly been achieved in human genomic studies (Belyeu et al. 2021) with call-quality (Duphold) filtering and automated curation of deletions using deep-learning (Samplot-ML), though these tools remain optimised for human data. Continued development and refinement of automated deep-learning methods for reliable curation of a broader range of structural variant classes in both short- and long-read data will prove to be of particular utility for future genomic studies on wild organisms.

Implications for downstream population genetic analyses of structural variants

Most population genetic studies of structural variants do not perform broad-scale visual curation on their SV callsets (Bruders et al. 2020; Catanach et al. 2019; Dorant et al. 2020; Liu et al. 2021; Rinker et al. 2019; Weissensteinter et al. 2020; Wold et al 2023). However, both our study

and previous studies (Belyeu et al. 2021; Bertolotti et al. 2020) have shown that rapid visual curation of SVs can easily detect and remove a large number of putative false positives. Even with the approach presented here (which excludes rare variants), provided larger sample sizes, prospective studies can easily inspect SV calls with allele frequencies at a standard MAF of 5% or less (**Fig. 1, 2**). Importantly, we found that larger SVs (>5 Kb) were both called at lower frequencies (**Table S2**) and prone to higher putative false positive rates (**Table 2**). In particular, the presence of false positive SV calls at lower frequencies (especially those called/genotyped at <20x coverage) could inflate the relative proportion of lower-frequency variants in e.g. site frequency spectra-based analyses. Therefore, studies inferring population genetic statistics from uncurated SV callsets may be biased by high false positives rates.

Before we can gain a detailed understanding of the population genetic nature of structural variants, a combination of both assemblies generated from high-accuracy long-read data (in the form of a pangenome graph) and population-level short-read re-sequencing data will be needed to expand the known range of SV variation in populations (Liao et al. 2023; Shi et al. 2023; Sirén et al. 2021; Wold et al. 2021). In particular, large SVs that vastly exceed the insert size range as well as those in highly repetitive regions are inherently harder to detect by both short-read mapping tools and manual visual curation. However, future developments could improve rare SV detection in wild populations, by leveraging manually-curated SV callsets as training data for A.I.-based detection methods (Cleal et al. 2022), as well as for use with other high-confidence callsets when constructing pangenome variant graphs (Sirén et al. 2021; Liao et al. 2023).

Conclusions

As important determinants of both deleterious and adaptive phenotypic effects, structural variants are increasingly targeted for evolutionary genomic studies of wild populations, including those of conservation concern. Such studies may often be constrained by computational resources or time, as well as funds to adequately re-sequence individuals to recommended coverage thresholds for short-read SV calling/genotyping (e.g. >20x to mitigate false-discovery rates and genotyping-errors; Wold et al. 2023) or to orthogonally validate putative true positive variants. We outline an easy and cost-effective strategy for enriching low-to-medium coverage short-read SV callsets with high-confidence variants, using only a single reference assembly. In complementing heuristic-based quality-filtering with rapid manual curation in a wild animal species, we demonstrate the feasibility of this approach in forming a part of short-read SV-detection pipelines. Prior to curation, the permissible putative false positive and false negative rates (lenient versus stringent curation strategies) may be chosen according to project goals (**Fig. 2**) and prospective curators trained accordingly. For example, a single curator applying a lenient curation strategy may suffice for population genomic studies attempting to characterise a broader pool of high-confidence SVs, while a stringent strategy applied by multiple curators may be necessary for functional validation studies or selecting probes for array-design. Given that a high-confidence SV catalogue generated from multiple long-read assemblies (e.g. a pangenome) will still be lacking for most genomic studies on wild-populations, time- and cost-effective filtering and curation of short-read-discovered SVs present an important alternative.

Materials and methods

Sampling and sequencing

DNA was collected from 33 house sparrow individuals from locations in Norway and Finland (see **Fig. S1** and **Table S1** for map and details of sampling sites, respectively). Blood samples were taken from the brachial vein and DNA was extracted as described in Hagen et al. (2013) using the ReliaPrep Large Volume HT gDNA Isolation System (Promega) automated on a Biomek NXp pipetting robot (Beckman Coulter). Samples were sequenced using a 100 bp paired-end Illumina TruSeq protocol with a short insert-size library of ~180 bp on 21 lanes on the HiSeq 2000 platform to a targeted average depth of ~10X (Elgvin et al. 2017). Adapter sequences were trimmed from raw reads using cutadapt v.2.3 (Martin 2011) with options "--minimum-length=30 --pair-filter=any". An overview of the number of reads for each sample before and after filtering is provided in **Table S5**.

Short-indel calling

Trimmed reads were aligned with BWA-MEM (bwa v.0.7.17) to the short-read reference genome assembly for *Passer domesticus* (Elgvin et al. 2017), GCA_001700915.1_Passer_domesticus-1.0), available at: (https://www.ncbi.nlm.nih.gov/assembly/GCA_001700915.1/) and then sorted and indexed with Samtools v.1.9. All unplaced scaffolds were removed and thus only scaffolds mapped to chromosomal regions were included in downstream analyses. Short indels were called and genotyped with GATK v.4.1.4.1 using the HaplotypeCaller and GenotypeGVCFs functions. Short indels were extracted from the resulting joint-called .vcf file with "SelectVariants -select-

type INDEL" and then filtered using "VariantFiltration" with the recommended filter expressions from GATK (McKenna et al. 2010) using: "QD < 2.0", "QUAL < 30.0", "FS > 200.0" and "ReadPosRankSum <- 20.0".

Large structural variant calling and genotyping

Among generalist structural variant callers for low- to medium-coverage short-read datasets, LUMPY performs with higher sensitivity compared to other common programs (Cameron et al. 2019). We therefore called larger (>20bp) structural variants (deletions, duplications and inversions) from the aligned .bam files using LUMPY (Layer et al. 2014) and genotyped the resulting calls with SVTyper (Chiang et al. 2015), via the smoove pipeline (Pedersen et al. 2020). This resulted in a file of genotyped structural variants (homozygotes reference, heterozygote or homozygote alternate) that was then queried with BCFtools (Danecek et al. 2021) for polymorphic structural variant calls with "bcftools query -f '%CHROM\t%POS\t%END\t%ALT[\t%GT]\n'" (Li et al. 2009).

Heuristic-based call- and genotype-filtering with Duphold and MSHQ

As per recommendations from Wold et al. (2023) and Pedersen and Quinlan (2019), we filtered raw callsets for call-quality and genotype-quality. We filtered deletions and duplications with Duphold (Pedersen and Quinlan 2019), a heuristic-based filtering tool that excludes suspected false-positives based on fold-change thresholds applied to regions 1 Kb in length adjacent to putative structural variants. We applied Duphold Flanking Fold-Change (DHFFC) thresholds by only retaining putative deletions with "DHFFC < 0.7" and duplications with "DHFFC > 1.3" in BCFtools. We additionally filtered all SV classes (deletions, duplications and inversions) with

Mean Smoove Heterozygote Quality scores provided by smoove, retaining only variants with a genotype-quality score above "MSHQ >= 3" (alternate variants with heterozygote individuals) or equal to "MSHQ=-1" (alternate variants with homozygote individuals only) in BCFtools.

Automated filtering with Samplot-ML

A deep-learning approach using Samplot-ML (Belyeu et al. 2021) was used to curate putative deletions with a convolutional-neural network algorithm adapted to Samplot. Samplot-ML is not yet available for automated curation of duplications and inversions.

Visual curation with Samplot/PlotCritic

Samplot (Belyeu et al. 2021) was used to generate .png files to visualise structural variants for manual visual curation (see Supplementary Materials). A custom Python script (gen_samplot.py) was used to select SVs represented by at least three individuals of the homozygote reference, homozygote alternate, and heterozygotes (see **Fig. 1**) and to generate plots of least two to three individuals per genotype. Occasionally one to two individuals were repeated in the same Samplot due to sampling with replacement, though the overall effect on curation was deemed to be at most negligible for the focus of this study. Juxtaposition of individuals from each of the three genotype classes increased both speed and accuracy during SV call curation. PlotCritic websites were established separately for each variant class (deletion, duplication or inversion) via an Amazon Web Services Instance, using commands provided through SV-Plaudit pipeline (Belyeu et al. 2018) (note: PlotCritic is now available independent of AWS (Belyeu et al. 2021)). All four curators were provided guidelines for identifying putative true positive structural variants, from features previously agreed upon by G.D. and A.B. In order to contrast different curation

strategies (e.g. lenient: remove obvious putative false positives; stringent: identify unambiguous putative true positives with only correct genotypes), each SV was then given a score of "Yes", "Maybe" or "No" during visual inspection by curators G.D., H.B., E.G., but only "Yes" or "No" by A.B..

Summary reports were downloaded from each PlotCritic website and curation scores were extracted. For each variant class, a .bed file of all variants receiving the score "Yes" was created for each curator. To create the final curated set of high-quality SVs, the .bed files across all 4 curators were intersected using BEDOPS v. 2.4.39 with the --intersect flag (Neph et al. 2012). The resulting .bed file was then intersected with the bcftools query .bed file for all SVs generated earlier, by specifying 90% reciprocal overlap "-f 0.9 -r" with BEDTools v2.29.2 (Quinlan and Hall 2010) to filter out SVs with largely redundant overlap. In this study, we define "putative true positive" (high-confidence) SVs as confirmed by one or more curators, while "putative false positive" SVs were rejected by one or more curators (Pedersen and Quinlan 2019). We note however that we have not functionally validated the SVs (see Discussion).

SV annotation and functional effect prediction

Curated SVs were annotated and their functional effect predicted using SnpEff v. 4.3t, with the putative impact defined as "low", "medium" or high" according to Cingolani et al. (2012). Partial and complete (100%) overlap of annotated genic regions (using available annotation for the GCA_001700915.1 genome (Elgvin et al. 2017) with curated SVs was determined with BEDTools v2.29.2 (Quinlan and Hall 2010). Size distributions were calculated from the curated SV bed files using the Pandas library (McKinney 2012) in Python3 (Van Rossum and Drake

2009). Curated SVs were also intersected with a repeat library (see below), using BEDTools v2.29.2 (Quinlan and Hall 2010).

Repeat library construction

Repetitive elements were identified using the Earl Grey TE annotation pipeline (version 1.2) (Baril et al. 2021, 2022), configured with Repbase (version 23.08) and Dfam (version 3.4) repeat libraries (Hubley et al. 2016; Jurka et al. 2005). Briefly, Earl Grey first annotated known repeats using the *Aves* repeat library. Following this, Earl Grey identified and refined novel TEs using an automated and iterative implementation of the "BLAST, Extract, Extend" process (Platt et al. 2016). Following final TE annotation, overlapping and fragmented annotations were resolved by Earl Grey before final TE quantification.

Population structure analyses

We compared the principal component analysis (PCA) using SVs, with the PCA of all genotype likelihoods for SNPs estimated with the GATK model "-GL 2, -doGlf 2 -SNP_pval 1e-6, -doMajorMinor 1 -doMaf 2 -minMapQ 30 -minQ 20" with ANGSD v. 0.921 (Korneliussen et al. 2014). Covariance matrices for the genotype likelihoods of SNP and SV callsets were extracted using PCAngsd (Meisner and Albrechtsen 2018), decomposed in R (R Core Team, 2020), with scripts from Mérot et al. (2023) and plotted with Python3 (Pedregosa et al. 2011). We then compared population structure recaptured with SVs both retained and rejected by curators to 1,200 and 15,000 randomly downsampled SNPs obtained from ANGSD with "-doGeno 4, -doPlink 2". Downsampling was performed using PLINK v1.90 (Purcell et al. 2007) with "--thin-

count" flag, with the subset of downsampled SNPs roughly equal to the number of loci in our callsets of the four-curator callset (1,243 deletions) and uncurated deletions (15,029).

Pairwise mean and weighted F_{ST} (as defined by Weir and Cockerham (1984)) was calculated using the --weir-fst option in VCFtools (version 0.1.16) for the following datasets: all ~30 x 10^6 raw SNPs, 1200 randomly downsampled SNPs, 1243 high-confidence deletions retained by all curators, 1004 deletions rejected after curation, 1135 deletions rejected after filtering with Duphold (DHFFC <0.7), and all raw unfiltered and uncurated deletions. Because a key population ("Pasvik") was represented by only two samples, we chose two individuals for each of the four major population clusters identified with the principal component analyses were chosen for F_{ST} comparisons: "Trøndelag": individuals 8L52141 and 8L52815; "Pasvik": 8L19747 and 8L19766; "Finland": FIN33 and FIN248; "Leka/Vega": 8L64093 and 8N73248 (see **Table S1**. for further sample location details).

Supplementary Material

Supplementary materials are available at *Genome Biology and Evolution* online.

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Author contributions

AHu conceived the study and AHu, GD, and RL designed the study. GD performed all bioinformatic analyses with the assistance of AB, DS and RL. AB, AHu, GD, EG, and HB conducted visual inspection of SVs. AHa and TB provided transposable element annotations and analyses. HJ provided the whole genome sequence data, map of sampling locations and sample details. GD and AHu wrote the first draft of the manuscript and all authors contributed to further versions.

Data Availability

The Illumina reads and assembled reference genome from this article are available at NCBI, Bioproject number PRJNA255814 (*P. domesticus* reference accession number SAMN02929199). Additional data and script are available at the Dryad database:

(Reviewer sharing link)

https://datadryad.org/stash/share/lik6XKWSVLN5pyVhIxOkcxrhDAqSH_sFNmEnSdTMwyo

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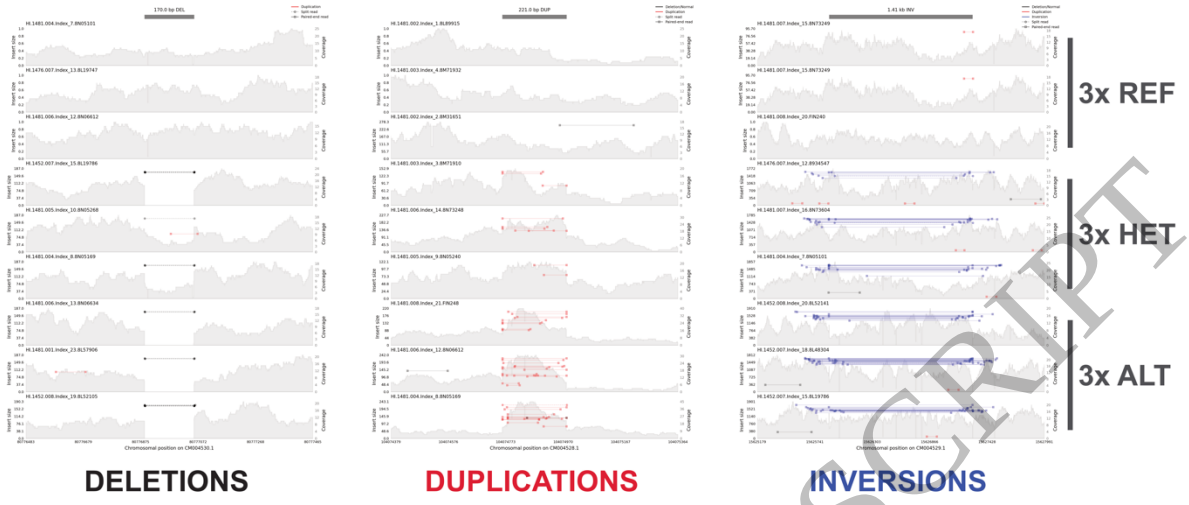
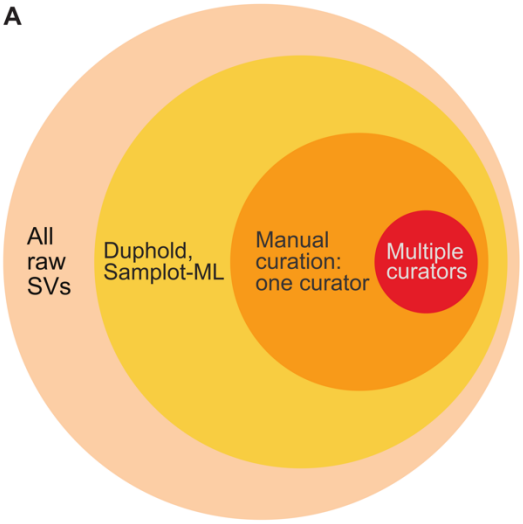


Figure 1. An example layout of Samplot-generated images in PlotCritic. The rapidity and efficiency of manual curation is greatly improved by leveraging the context of individuals with differing genotypes in a fixed order, in this case 3 homozygote reference individuals, 3 heterozygote individuals, and 3 homozygote alternate individuals. Here 3 putative true positive structural variants are shown, each plotted for 9 individuals representing all three genotypes. Note that fewer individuals per genotype may be visualised than shown here, allowing for curation of lower-frequency variants.

A



B

Filtering	% of SVs removed	Number of curators	Application
None	n/a	n/a	Not recommended
Duphold, Samplot-ML	~10-25%	n/a	Population genomics
Manual: Samplot/PlotCritic	~30-80%	One	Population genomics for large sample sizes; functional genomics
Manual: Samplot/PlotCritic	~65-95%	Multiple	Functional genomics; probes for array-design

Figure 2. *(A)* The tradeoff between the proportion of rejected SVs (deletions, duplications and inversions) following heuristic-based filtering and manual curation, versus the increasing confidence that SVs may represent putative true positives. The proportion of retained SVs after each filtering step is represented by circles; smaller circles indicate a decreasing number of retained SVs, while darker colour indicates increasing confidence in SV calls and genotypes. *(B)* Summary of filtering methods, proportion of SVs removed, number of manual curators required and downstream applications for SV callsets of varying confidence. Percentages for one and four curator callsets are fractions of the "genotype-frequency filtered" subset (SVs selected for curation which are represented by at least three individuals of each genotype class). Note that automated curation with Samplot-ML is currently only possible for deletions.

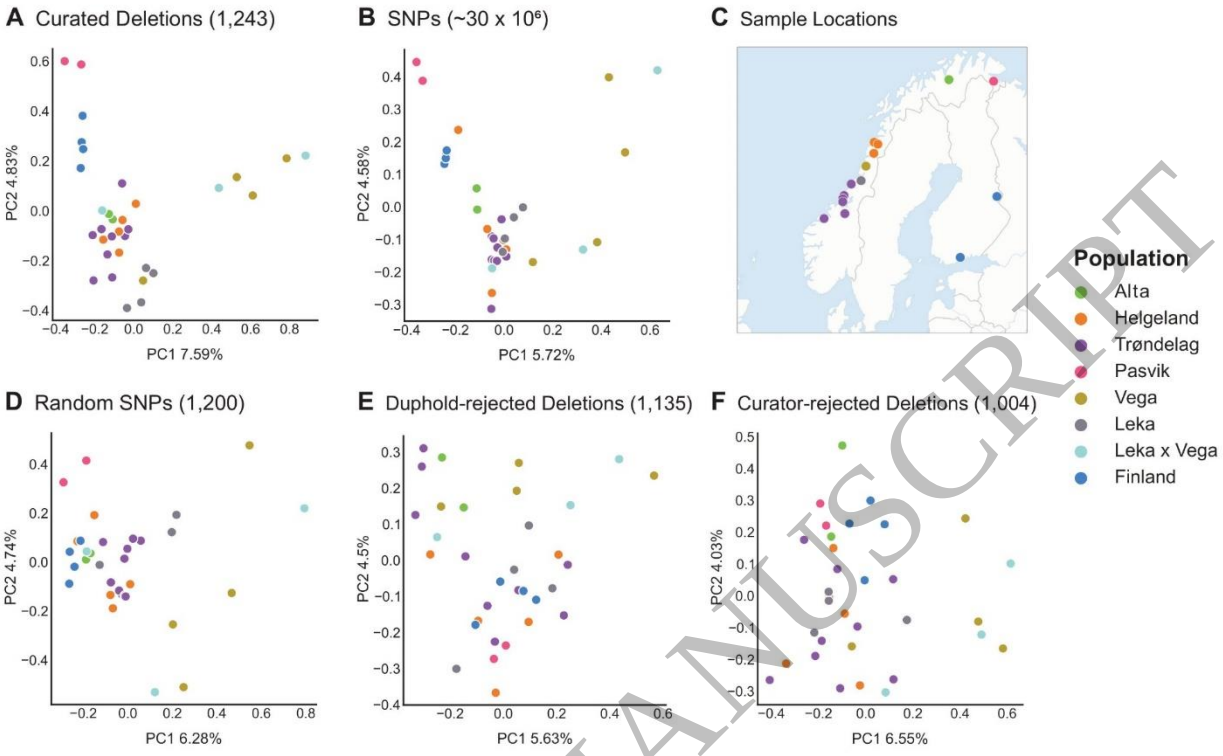


Figure 3. PCAs showing the effect of manual curation with Samplot/PlotCritic and filtering with Duphold on raw SV calls, in recapturing expected population structure. *(A)* 1,243 Deletions retained by complete agreement between all four curators (strategy minimising the false positive rate; least number of putative false positives retained), *(B)* all 30,275,406 raw SNPs, *(C)* Sampling locations for all 33 individuals, *(D)* 1,200 randomly downsampled SNPs, *(E)* all 1,135 Deletions rejected by Duphold filtering (DHFFC <0.7), *(F)* all 1,004 Deletions rejected by the most lenient curator (strategy minimising the false negative rate; least number of putative true positives rejected). Rejected deletions shown in *(F)* were also rejected by near-complete (>99%) agreement between all four curators.

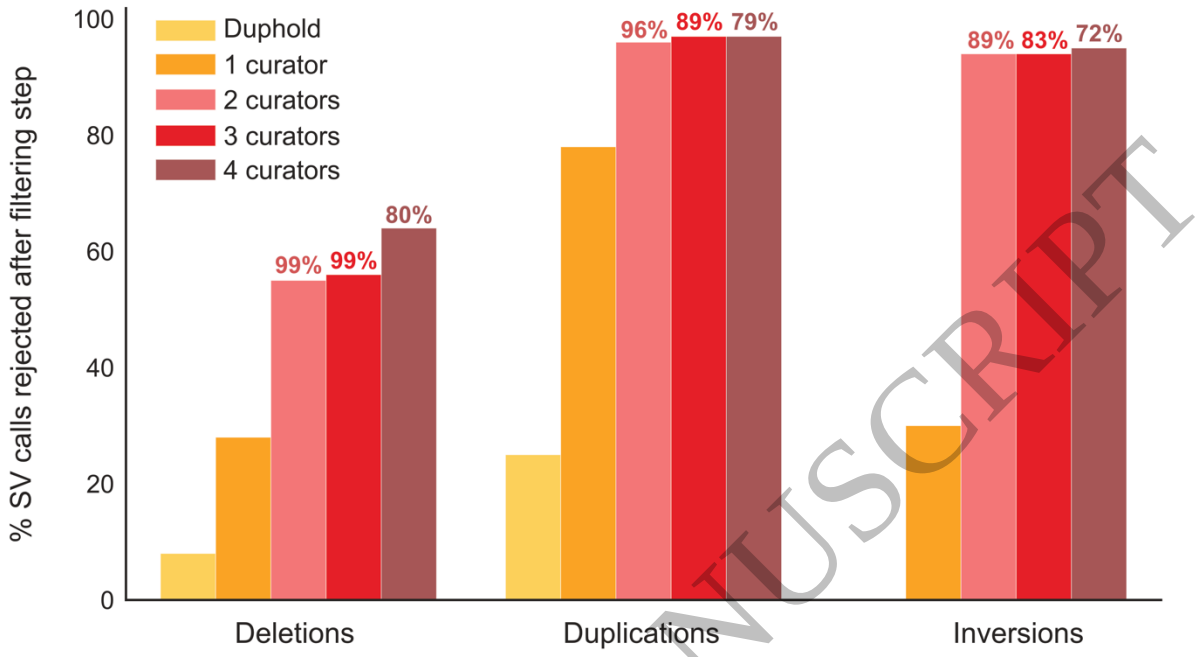


Figure 4. % SV calls rejected after each filtering step, per SV class. Values above the barplots indicate the percent "agreement" between all other curators versus the strictest curator: the intersect between variants rejected by the strictest curator versus those rejected by other curators. Curators are added in increasing order of stringency, where 1 curator = the most lenient curator (rejecting the fewest variants) and 4 curators includes the most stringent curator (rejecting the most variants). Percent rejected SV calls for one and four curator callsets are fractions of the "genotype-frequency filtered" subset (SVs selected for curation which are represented by at least three individuals of each genotype class). Note: Duphold (filtering by fold-change in variant coverage) only applicable for deletions and duplications.

Table 1. Size range (in bp) for raw, filtered and curated SV classes. Counts for one-curator (the most lenient curator; strategy rejecting the fewest variants) and four-curator callsets are fractions of the subset filtered by "genotype-frequency" (SVs selected for curation which are represented by at least three individuals of each genotype class; see table S2). Note: Samplot-ML only applicable for deletions; Duphold (filtering by fold-change in variant coverage relative to flanking regions) only for deletions and duplications.

Curation	Maximum length	Minimum length	Mean length	Median length	Count
Deletions					
Raw, uncurated	142,501,103	23	319,153	376	15,029
Samplot-ML	142,501,103	23	185,764	347	14,345
Duphold	81,855,813	23	110,402	333	13,894
One curator (lenient)	6,096	25	257	106	2,457
All four curators	6,096	25	260	109	1,243
Duplications					
Raw	120,312,679	79	1,497,613	1350	3,430
Duphold	120,312,679	79	917,802	625	2,560
One curator (lenient)	2,121,873	95	13,290	345	287
All four curators	411	98	162	126	37
Inversions					
Raw, uncurated	76,386,443	33	639,090	83	1,188
One curator (lenient)	2,057	37	87	376	177
All four curators	107	49	68	63	13

Table 2. Relative proportions (%) of putative false positive variants rejected by each filtering step. Percentages for one-curator (the most lenient curator; strategy rejecting the fewest variants) and four-curator callsets are fractions of the subset filtered by "genotype-frequency" (SVs selected for curation which are represented by at least three individuals of each genotype class; see table S2). Note: Samplot-ML only applicable for deletions; Duphold (filtering by fold-change in variant coverage) only for deletions and duplications.

Filtering Method	>20 to 100 bp	>100 to 250 bp	>250 to 500 bp	>500 bp to 1 Kb	>1 to 5 Kb	>5 to 10 Kb	>10 to 500 Kb	>500 Kb	Percent Rejected (Total variants removed)
Deletions									
Samplot-ML	**0.4**	**0.6**	**1.9**	**2.5**	**6.6**	**6.3**	**27.5**	**45.5**	**4.6** (684)
Duphold	**0.8**	**2.5**	**4.0**	**5.5**	**11.4**	**6.7**	**35.9**	**67.0**	**7.6** (1,135)
One curator (lenient)	**7.9**	**12.2**	**47.8**	**58.6**	**56.1**	**96.0**	**100.0**	**100.0**	**29.0** (1,004)
All four curators	**54.4**	**51.1**	**77.1**	**80.1**	**76.0**	**98.0**	**100.0**	**100.0**	**64.1** (2,218)
Duplications									
Duphold	**0.0**	**0.9**	**5.4**	**20.6**	**32.5**	**48.8**	**47.0**	**41.9**	**25.4** (870)
One curator (lenient)	**50.0**	**47.7**	**70.8**	**87.3**	**59.5**	**93.9**	**99.0**	**98.9**	**77.6** (2,149)
All four curators	**90.0**	**85.6**	**97.8**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**97.1** (3,393)
Inversions									
One curator (lenient)	**23.9**	**30.8**	**50.0**	**66.7**	**0.0**	**100.0**	**100.0**	**100.0**	**30.0** (75)
All four curators	**94.4**	**92.3**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**94.8** (239)