Esterase-5 gene sequences from the *Drosophila buzzatii* cluster species.

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Introduction

The autosomal gene Esterase-5 (Est-5) was described as a possible member of the carboxyl/cholinesterases gene family (Cygler *et al.,* 1992; Hemilä *et al.,* 1994; Oakeshott *et al.,* 1999, 2005). The members of this gene family have high evolutionary rates and low amino acid identity probably due to their diversification in DNA sequence and biochemical function, relative to the ecological and behavioral changes of the hosts species (Oakeshott *et al.,* 1999, 2005). In *Drosophila spp t*wo groups of carboxyl/cholinesterases were described: α-esterases and β-esterases, associated to the hydrolyse of α and β-naftil-acetate, respectively. The α-esterase group was characterized from *Drosophila melanogaster* (Robin *et al.,* 2000). In *D. buzzatii* species the Esterase-5 gene encodes possibly the Esterase-2 protein that is homologous to Esterase-9 from *D. melanogaster* (Oakeshott *et al.,* 1993; East *et al.,* 1990). That protein is expressed in digestive trait and Malpighi tubes in *D. buzzatii*, indicating a possible relationship with alimentary behavior (East *et al.,* 1982); however, the true function is still unknown (East *et al.,* 1990).

The *D. buzzatii* cluster (*D. repleta* group: *D.buzzatii* complex) is a monophyletic group (Ruiz *et al.,* 1993; Manfrin *et al.,* 2001), composed by seven species: *D. buzzatii, D. serido, D. antonietae, D. seriema, D. gouveai, D. borborema,* and *D. koepferae*, and the main diagnostic characteristic is the aedagus morphology (Silva and Sene, 1991). They are endemic of the South American continent, with the exception of *D. buzzatii* that was introduced in other continents (Barker *et al.,* 1985). The species of this cluster are cryptic and cactophilic, whose larvae feed exclusively on decayed tissues of cacti (Pereira *et al.,* 1983). The distribution of the *D. buzzatii* cluster species ranges from Northeast region of Brazil to Chaco Domain in Argentina and is associated with different species of cacti (Manfrin and Sene, 2006). The cladogenetic events in this cluster are associated to pre-Quaternary vicariant events (Manfrin *et al.,* 2001; De Brito *et al.,* 2002), and the population structure and diversification are associated to the retraction and expansion events of the dry vegetation (caatinga and savanna like vegetations) and moist vegetation (rainforest like vegetations) of the South America, during the paleoclimatic changes, as the Quaternary glaciations (Sene *et al.,* 1988).

Historical events, ecological interactions, and genetic characteristics of the species are fundamental to understanding the current patterns of distribution, species diversification, and population variation. For the *Drosophila buzzatii* cluster a lot of historical information is available; however, little information exists regarding the genes associated with ecological interactions, such as the adaptation to the use of decaying tissues of cactus to feed their larvae. Thus, this study aimed to evaluate information in sequences of the Esterase-5 gene in species of the *D. buzzatii* cluster for future works.

Material and Methods

We extracted DNA from 18 individuals of natural populations of the species of the *D. buzzatii* cluster: *D. buzzatii* (2 individuals), *D. koepferae* (3 individuals), *D. borborema* (1 individual), *D.*

serido (3 individuals), *D. seriema* (3 individuals), *D. antonietae* (3 individuals), and *D. gouveae* (3 individuals). For DNA extraction, we used the Puregene kit (GENTRA Systems). We performed the PCR reaction using three sets of primers (Table 1), designed from *D. borborema* sequence from GeneBank (DQ453800) (Figure 1). The PCR reaction conditions were: 1 µl DNA template, 5 µl Buffer, Magnesium Chloride free, 3 µl Magnesium Chloride (2mM/ µl), 4 µl dNTP (4 pmol/µl), 2 µl of each primer (5 pmol/ µl), 0.2 µl Taq polymerase (5 U/ µl) and 32.8 µl of water in a final volume of 50 µl per reaction. The thermocycler program used was one cycle of 94°C, 4 minutes; 35 cycles of 94°C, 40 seconds, 53°C, 40 seconds, 72°C, one minute, and 72°C 3 minutes, and a final extension of 4°C without limit of time. The sequencing reaction was performed directly from the PCR fragments following the protocol in Piccinali *et al.* (2007). The sequences were checked by eye using the program Chromas lite v 2.0 (Free Software from: http://www.technelysium.com.au/chromas_lite.html, access: 18/12/2009) and aligned with Piccinali *et al.* (2007) sequences and *D. virilis* (CH940652) and *D. mojavensis* (CH933806) from Gene Bank using Bioedit program v. 7.0.9.0 (Hall, 1999). To check for possible phylogenetic information, we performed a Maximum Parsimony test (10000 replications) in PAUP v 4.0 program (Swofford, 2001).

Table 1. Characteristics of the primers used to amplify the fragments of Esterase-5 gene.

Name of the primer	Sequence	Size (bp)	Molecular Weight	Annealing temperature °C
Ester-2F	TGG ACT GAA GGA CCA GGT TT	20	6197.1	57.3
Ester-2R	CTT GTC GTA CTC GGG CAG AT	20	6124.1	59.4
Ester-CH1 F	GGG CAC CTT GCA CAA CTA TT	20	6058.0	57.3
Ester-CH1R	AGC CAT GCC AGA AGA TCC TA	20	6076.0	57.3
Ester-CH2F	ATA TG TGT GCA CGG AGC AG	20	6187.0	57.3
Ester-CH3R	CTT CAT CTT CTT GCT GGA CT	20	6006.0	55.3

Results and Discussion

We tested all sets of primers (Table 1) and the combinations of these primers to produce DNA sequences that cover at least the exon 4 and 5 from the Esterase-5 gene. In the first test we used the set of primers Ester-2F and Ester-2R (Figure 1) that amplified a DNA fragment with approximately 1158 bp in all seven species. The set Ester-2F and Ester-2R cover a big portion of the exon 4 and 5 from the Esterase-5 gene. However, the sequences from the species *D. antonietae* and *D. gouveae* showed low quality of the sequences and some samples of the species *D. seriema, D. antonietae, D. gouveae, D. koepferae,* and *D. buzzatii* do not show PCR products. A second test was performed to produce an internal fragment with a better quality of the sequences. We used the set Ester-2F and Ester-CH1R that showed amplified products of 781 bp (Figure 1). However, these products showed low concentration of the PCR products in the species *D. antonietae, D. gouveae,* and *D. buzzatii,* and we made a re-amplification to produce PCR products in high concentration to perform the DNA sequence reaction. The re-amplified products do not show ghost bands, and the concentration was enough to perform sequencing reactions in all species. In a third test we used the set Ester-CH2F and Ester-2R to cover the final portion of the Esterase-5 gene and the exon 5. This set of primers produced fragments with 1039 bp, but can only be obtained from the species *D. borborema, D. seriema, D. koepferae,* and *D. buzzatii.* Even performing re-amplification and changing the PCR conditions, DNA sequences from the species *D. antonietae* and *D. gouveae* could not be obtained. The other combinations of the primers (Ester-2F and Ester-CH3R, Ester-CH2F and Ester-CH1R,

Ester-CH2F and Ester-CH3R, Ester-CH1F and Ester-2R, Ester-CH1F and Ester-CH1R, Ester-CH1F and Ester-CH3R) did not produce DNA fragments even with modifications in the PCR conditions. The consensus sequence sizes varied from 614 bp in the *D. antonietae,* 740 bp from *D. gouveae,* 888 bp from *D. borborema,* 1032 bp from *D. serido*, 1062 bp from *D. kopferae,* 1085 bp from *D. seriema,* and 1091 from *D. buzzatii* (see Appendix 1).

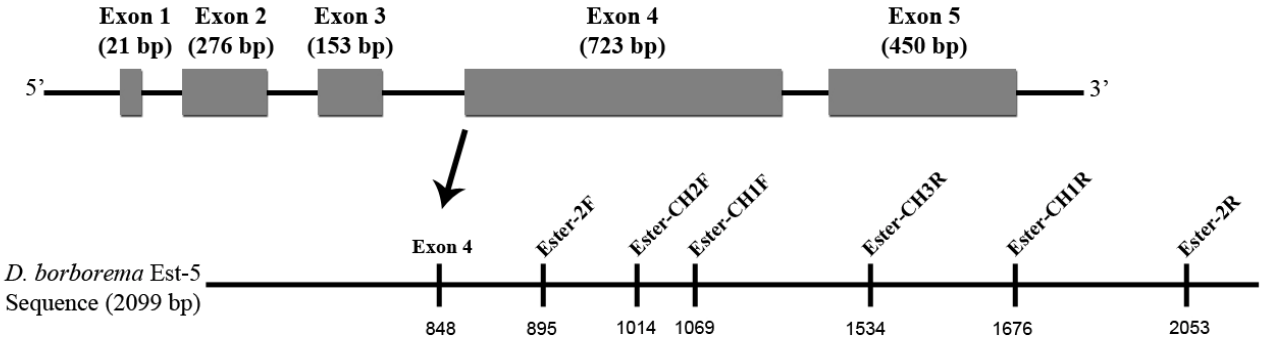

Figure 1. Location of the primers, based on the *D. borborema* sequence from GeneBank, used in this work. In the top of the figure, representation of Esterase-5 gene and exons positions. In the low position of the figure, primer location, the numbers represent the position of the primers in base pairs, related of the *D. borborema* from GeneBank.

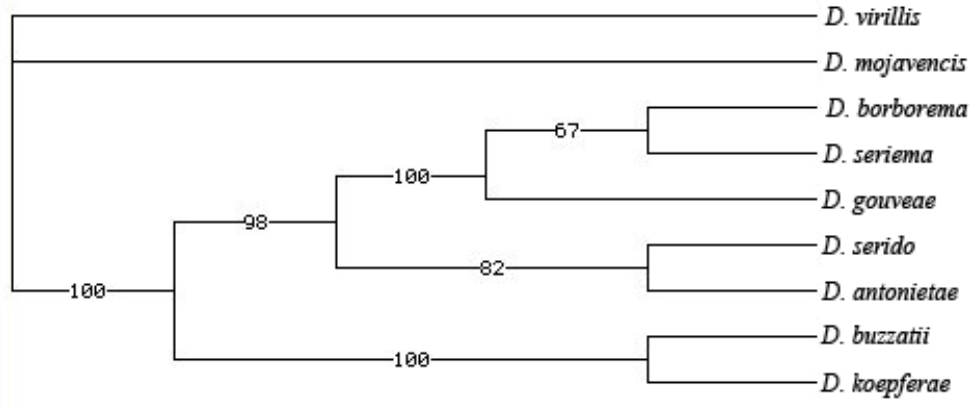

Figure 2. Maximum Parsimony tree based on the consensus sequence of the species used in this work. *D. virilis* and *D. mojavencis* was used as a outgroup. Values of bootstrap calculated based on 10000 replications.

The Maximum Parsimony analyses showed that the species of the *D. buzzatii* form a clade (booststrap values of 100%), separated from the external species *D. virillis* and *D. mojavencis* (Figure 2). The species in the cluster formed two clades. One encompassed *D. buzzatii* and *D. koepferae* (bootstrap values of 100%). The second one encompassed the other five species with bootstrap values of 98%. This second clade is divided in two groups: one with *D. serido* and *D. antonietae* species (bootstrap values of 82%) and the second group with the species *D. borborema*, *D. seriema,* and *D. gouveae* (bootstrap values of 100%). This phylogenetic hypothesis using the Esterase-5 gene

information is very similar with the ones elaborated using mitochondrial DNA (Manfrin *et al.,* 2001) and chromosomal inversions (Ruiz and Wasserman, 1993; Ruiz *et al.,* 1996). The similarity between the phylogenies obtained with the Esterase-5 gene and the other phylogenies (Manfrin *et al.,* 2001; Ruiz and Wasserman, 1993; Ruiz *et al.,* 1996) seems to be related to the historical relationship with the species of the *D. buzzatii* cluster; even the Esterase-5 showed many indications that it is a gene related to adaptative functions (East *et al.,* 1982). In this work we generated sequences from the Esterase-5 gene from the seven *D. buzzatii* cluster species, and it is a first step to perform future work. The genetic sequences are the base to develop new specific primer sets to be used in molecular systematics, phylogeography, adaptation, and ecological works and more.

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Appendix 1. Sequences aligned by the program Bioedit v 7.0.9.0 (Hall, 1999) using Blossun 62. * DNA Sequence of *D. borborema* from GeneBank (DQ453800). ** DNA Sequence from Robin (2000).

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                    10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
D. bor *        ATGTCAGAAAGGTGTTTGTTTTCAATAAAAACATACATTTAAATTCCATT
D. bor          --------------------------------------------------
D. buzz **      --------------------------------------------------
D. buzz         --------------------------------------------------
D. koep         --------------------------------------------------
D. anto         --------------------------------------------------
D. gouv         --------------------------------------------------
D. serido       --------------------------------------------------
D. serie        --------------------------------------------------
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                  60        70        80        90       100
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATCATAAATTAATGTGAATTTAATAATAATTTCATTTCATTTAGTTTGGA
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 110       120       130       140       150
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GAGCTGTGAAATATCATTGCCCGTGGGCCAAATCCGGGGCGTTAAGCGCC
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 160       170       180       190       200
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGCCTTTACGATGATGATTACTATAGCTTCGAGCGTTTGCCCTTTGGC
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 210       220       230       240       250
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AAGCCGCCAGTGGGTGAACTACGCTTTAAGGCACCTGTGCCAGTCGAGCC
D. bor      ---------------------------------------------------
D. buzz **  -------------TACACTGCGCTTTAAGGCACCTGTGCCGGTTGAGCC
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 260       270       280       290       300
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CTGGTCCGGTGTGCTGGACTGCACCCACTTTGCCGAAAAGCCCGTGCAGA
D. bor      ---------------------------------------------------
D. buzz **  CTGGTCCGGTGTGCTGGACTGCACCCACTTTGCAAATAAGCCGGTGCAGA
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------
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                 310       320       330       340       350
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGGGCTTGTTAACAGGCATCATAGAGGGCAGCGAGGACTGCCTTTATTTG
D. bor      ---------------------------------------------------
D. buzz **  AGGGCTTGCTAACGGGCATCATAGAAGGCAGCGAGGACTGCCTTTATCTG
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 360       370       380       390       400
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AATGTATACGCCAAACAGGTGACTTGCTAAAAAATAATATATCACTTTGA
D. bor      ---------------------------------------------------
D. buzz **  AATGTATACGCCAAACAGGTGACTTGCTAAAAAATAATATATCACTTTGA
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 410       420       430       440       450
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TGGATTGTTCAAATGTTCAAGTGATCTCACTGATAACAGCTGAAGAGCGC
D. bor      ---------------------------------------------------
D. buzz **  TGGATTGTTCAAATTTCCAAGTGATTTCACTGATAACAGCTGAAGAGCGC
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 460       470       480       490       500
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AAAACCCCTACCTGTTATGGTCTATATATACGGAGGCGCTTTCTCAATAG
D. bor      ---------------------------------------------------
D. buzz **  GAAACCATTACCTGTTATGGTCTATATATACGGAGGCGCTTTCTCCATAG
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 510       520       530       540       550
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCGAGGCCACTCGGGATATCTACTCGCCCGATTATTTTATGGCTAAAGAT
D. bor      ---------------------------------------------------
D. buzz **  GCGAGGCCACTCGGGAGATCTACTCGCCCGATTACTTTATGGCTAAAGAT
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------
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                  560       570       580       590       600
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      GTGGTTCTAGTCACCCTCAACTATCGCGTTGATTGTCTAGGTAAGTGATT
D. bor        ---------------------------------------------------
D. buzz **    GTGGTTCTAGTCACCCTCAATTATCGCGTTGATTGCCTAGGTAAGTGATT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  610       620       630       640       650
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      GTAAACTTATCGTTTGATTATCTCTTAAATAAGTTAGTTATATTAGTTGT
D. bor        ---------------------------------------------------
D. buzz **    GTAAACTTATCGTTTGATTATCTCTTAAAGAAGTTTGTTATATTAGTTGT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  660       670       680       690       700
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      TCAGCTAGTTTCTCTTATCAATTTGGGTATAAAGTTCGGCCTAGCTTTAA
D. bor        ---------------------------------------------------
D. buzz **    TTAGCTAGTTTCTCTTATCAATGTGGGTATAAAGTTCCGGCTAGCTTTAA
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  710       720       730       740       750
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      ATTATTTTTT-TGGTAAAAGCTTTTTCTCTTGCTCCCCTAGCTAAGCTTT
D. bor        ---------------------------------------------------
D. buzz **    GTTATTTTTTATGGCAAAAGCTTTTTCTCTTGCTCCCCTAGCTAAGCTTT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  760       770       780       790       800
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      CGCACTCAGCGTACATTCGAACTTGCTATTTTCACTCGTTTTCTCTCTCT
D. bor        ---------------------------------------------------
D. buzz **    CGCACTCAGCGTACATTCGGACTTGCTGTTTTCACTCTCTCTCTCTCTCT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------
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                 810       820       830       840       850
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       CTCTCTTTCTCTTTTCTCTTTTCTTATTTCCTTTTTCTT-----TTTCTT
D. bor         ---------------------------------------------------
D. buzz **     CTCTTTCACCGTTTCCCCCTCTTTCTTTCTCCTTTTATTCCATTTCTCTT
D. buzz        ---------------------------------------------------
D. koep        ---------------------------------------------------
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       ---------------------------------------------------

                 860       870       880       890       900
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       TAAGGTTTCCTTAGTCTAAAAGACCCCAGTTTGGAGGTGCCCGGAAATGC
D. bor         ---------------------------------------------------
D. buzz **     TAAGGTTTCCTTAGTCTAAAAGACCCCAGCTGTGAGGTGCCGGGAAATGC
D. buzz        ---------------------------------------------------
D. koep        ---------------------------------------------------
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       ---------------------------------------------------

                 910       920       930       940       950
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       TGGACTGAAGGACCAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. bor         ---------------------------------------------------
D. buzz **     TGGGCTAAAGGATCAGGTGTTAGCTATCAAATGGGTAAATCAATATATTT
D. buzz        ------------CAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. koep        ----------GACCAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       -----------------TTTTAGCTATCAAATGGGTAAATCAATATATTT

                 960       970       980       990       1000
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTTGGTGAAAGCGCC
D. bor         ---------------------------------------------------
D. buzz **     CCTATTTCAATGGTGCTGTGAACAACATAACGGTGTTCGGTGAAAGCGCC
D. buzz        CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTCGGTGAAAGCGCC
D. koep        CCTATTTCAATGGTGATGTGAACAACATAACGGTATTCGGTGAAAGCGCC
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------GTTTGGTGAAAGCGCC
D. serido      -----------GGTGATGTGAAGAACATAACGGTGTTTGGTGAAAGCGCC
D. serie       CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTTGGTGAAAGCGCC

                 1010      1020      1030      1040      1050
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACGCGCGGACT
D. bor         ----------------------------ACGGAGCAGACACGTGGACT
D. buzz **     GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. buzz        GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. koep        GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. anto        ----------------------------ACGGAGCAGACGCGTGGACT
D. gouv        GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACACGTGGACT
D. serido      GGCGGCTGTTCCACCCACTATATGATGTGCACAGAGCAGACGCGTGGACT
D. serie       GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACACGTGGACT
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                1060      1070      1080      1090      1100
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. bor      CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. buzz **  CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. buzz     CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. koep     CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. anto     CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. gouv     CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. serido   CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAATTATTGGTCCA
D. serie    CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA

                1110      1120      1130      1140      1150
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATACC-CCTCCCGCAGACTTTGCCTATCGACTGGCAAA-GGGGAATGGGT
D. bor      ATACG-TCGCCCGCAGACTTTGCCTATCGTCTGGCCAA-GTTGAATGGGT
D. buzz **  ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. buzz     ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. koep     ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. anto     ACACGGCCNNCCGCAGACTTTGCCTATCGTCTGGCCAAAGGTGAATGGGT
D. gouv     ATACC-CCGCCCGCAGACTTTGCCTATCGACTGGCCAA-GGTGAATGGGT
D. serido   ACACGGC-GCCCGCAGACTTTGCCTATCGTCTGGCCAA-GGTGAATGGGT
D. serie    ATACC-CCTCCCGCAGACTTTGCCTATCGTCTGGCCAA-GGTGAATGGGT

                1160      1170      1180      1190      1200
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. bor      ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. buzz **  TTGAAGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. buzz     TTGAAGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. koep     TTGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. anto     ATNAGGGCTGAAAACAATGATCGCCAGGTGCTGNACTATTTGCGCACTGT
D. gouv     ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. serido   ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. serie    ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT

                1210      1220      1230      1240      1250
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CCCAGCAGAGCAGTTGGTTAACCACAGCCTGCTCACTCCT-GAGGATCGG
D. bor      GCCGGCAGAGCAGTTGGTTAACCACAGCCTGCTCACTCCTTGAGGATCGG
D. buzz **  GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAAGATCGG
D. buzz     GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAGGATCGG
D. koep     GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAGGATCGG
D. anto     GCCGGCAGAGCAGCTGGTTAGCCACACCCTGCTCACCCCT-GAGGATCGG
D. gouv     ACCAGCAGAGCAGCTGGTTAACCACAGCCTGCTCACACCT-GAGGATCGG
D. serido   GCCGGCAGAGCAGCTGGTTAGCCACAGCCTGCTCACCCCT-GAGGATCGG
D. serie    GCCGGCAGAGCAGTTGGTTAACCACAGCCTGCTCACCCCT-GAGGATCGG

                1260      1270      1280      1290      1300
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CGCACCGGACTTATTTATGCCTTTGGCCCCACCGGGGAGCCATACGTGAT
D. bor      CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. buzz **  CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAACCATACGTGAT
D. buzz     CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. koep     CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. anto     CGNAACGGACTCATCTATGCNTTTGGCCCCACCGTGGAGCCATACGTGAT
D. gouv     CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. serido   CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. serie    CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
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                1310      1320      1330      1340      1350
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGTAGACTGTGTGGCTCCAAAGCCACAGTTGGAAATGGTGCGCAATGCTT
D. bor      GGTAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT
D. buzz **  GACAGACTGTGTTACTCCAAAGCCACAGCTGGAGATGGTGCGCAAAGCTT
D. buzz     GGCAGACTGTGTAGCTCCAAAGCCACAGCTGGAGATGGTGCGCGAAGCTT
D. koep     GGCAGACTGTGTAGCTCCAAAGCCACAGCTGGAGATGGTGCGCGAAGCTT
D. anto     GGTAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT
D. gouv     GGTAGACTGTGTGGCTCCAAAACCACAGCTGGAGATGGTGCGCGATGCTT
D. serido   GGTAGACTGTGTGGCTCCAAAGCCACAGCTAGAGATGGTGCGCGATGCTT
D. serie    GATAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT

                1360      1370      1380      1390      1400
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGAGCAATAAGTTACCAGCCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. bor      GGAGCAATAAGTTACCAGTCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. buzz **  GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACTTCTTTCGAAGGTCTC
D. buzz     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. koep     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. anto     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. gouv     GGAGCAATAAGTTACCAGCCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. serido   GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. serie    GGAGCAATAAGTTACCAGTCATGCTGGGCGGCACCTCTTTCGAAGGTCTC

                1410      1420      1430      1440      1450
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TTCATGTATCCCGCCTTGAAGGCTAATCCTAAGGGCATGGATAGCCTGCC
D. bor      TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. buzz **  TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. buzz     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGAGCATGGATAGCCTGCC
D. koep     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. anto     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. gouv     TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. serido   TTCATGTATCCCGCCTTGAAGGCCAATGCTAAGGGCATGGATAGCCTGCC
D. serie    TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC

                1460      1470      1480      1490      1500
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGGATCTGCTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. bor      GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. buzz **  GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. buzz     GCAGGATCTCTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. koep     GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. anto     GCAGGATCTGTTGCGATTAACGCCCTATGAAGTGCGTGTGCTCAATAAGG
D. gouv     GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. serido   GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. serie    GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG

                1510      1520      1530      1540      1550
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. bor      AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. buzz **  AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. buzz     AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. koep     AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. anto     AACAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. gouv     AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. serido   AACAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. serie    AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
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                1560      1570      1580      1590      1600
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GATGACACGCCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGTT-
D. bor      GATGCCACGCCCAGCTCCAAGTTCATCATGAACTTCATGGACGTGAGCTG
D. buzz **  GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. buzz     GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. koep     GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. anto     GATGCCACGCCCAGCTCCAAGCTCATCATGAACTTTATGGACGNNNNTN-
D. gouv     GATGACACGCCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. serido   GATGCCACGCCCAGCTCCAAGCTCATCACGAACTTCATGGACGTGAGTT-
D. serie    GATGCCACCCCCAGCTCCAAGTTCATCATGAACTTCATGGACGTGAGTT-

                1610      1620      1630      1640      1650
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTAT----
D. bor      CGAATCACTTAAGTTTAACTATATAACTGTACTCTA-ATTCTTTAT----
D. buzz **  CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. buzz     CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. koep     CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. anto     NNNATCACTTAAGTTTAATTATATACT-GTACTCTATATACTGTACT---
D. gouv     CGAATCACTTAAGTTTAACTATATAACTGTACTCTA-ATTCTTTAT----
D. serido   CGAATCACTTAAGTTTAACTATATATT-GTACTCTATATACTGTACTCTA
D. serie    CGAATCACT----TTTAACTATATAACTGTACTCTA-ATTCTTTAT----

                1660      1670      1680      1690      1700
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. bor      ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT
D. buzz **  ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. buzz     ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. koep     ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. anto     --------------------------------------------------
D. gouv     ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT
D. serido   ATTCTTTATATTTCTACTTTTAGTACTATTCCTATAGGATTTTCTGGCAT
D. serie    ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT

                1710      1720      1730      1740      1750
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCCCCCAC
D. bor      GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCCCCCAC
D. buzz **  GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. buzz     GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. koep     GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. anto     --------------------------------------------------
D. gouv     GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGG-------
D. serido   GGCTTCCATCGTACTTTGCAAGCTCGCCTGGCATACGCCACGGCTCCCAC
D. serie    GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCTCCCAC

                1760      1770      1780      1790      1800
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TTACTTCTATCGCTTTGATTTTGATTCACCTGGTTTCAATTTCTACCGTA
D. bor      TTACTTCTATCGCTTTGATTTTGATTCACCTGGTTTCAATTTCTACCGTA
D. buzz **  ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. buzz     ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. koep     ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   TTACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTTCCGTA
D. serie    TTACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
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                1810      1820      1830      1840      1850
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. bor      AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. buzz **  AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTGGCCCATGCCGATGA
D. buzz     AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. koep     AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   AGAAATTCTGCTGGCGATGACATTAAGCAGGGCGTTGCCCATGCCGATGA
D. serie    AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA

                1860      1870      1880      1890      1900
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCAT
D. bor      GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCAT
D. buzz **  GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. buzz     GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. koep     GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTAGACAAAGCCT
D. serie    GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCCT

                1910      1920      1930      1940      1950
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CCGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. bor      CCGCTGAATATCGCACCATTCAACGGCTGGTCGACA--------------
D. buzz **  CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. buzz     CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. koep     CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   CCGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. serie    CCGATGAATATCGCACCATTCAACGTCTGGTCGACATTTGGACATCCTTT

                1960      1970      1980      1990      2000
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGCA
D. bor      --------------------------------------------------
D. buzz **  GCAGCTACTTCCAATCCCAACTGCGCT-----------------------
D. buzz     GCAGCTACTTCCAATCCCAACTGCGCTGAGACTGCGCATCTCGATTGGCA
D. koep     GCAGCTACTTCCAATCCCAACTGCGCTGAGACTGCGCATCTCGA------
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGC-
D. serie    GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGCA

                2010      2020      2030      2040      2050
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCCAGCTAAGCAGGAGCAGCCTCAGCGTGTGCTCAACATTGGCAAAGAGG
D. bor      --------------------------------------------------
D. buzz **  --------------------------------------------------
D. buzz     GCCAGCGAAGCAGGAGCAGCCTCAGC-----------------------
D. koep     --------------------------------------------------
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   --------------------------------------------------
D. serie    GCCAGCTAAGCAGGAGCAGCCTCAGC-----------------------
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                2060      2070      2080      2090      2100
           ....|....|....|....|....|....|....|....|....|....|
D. bor *   TGGAGCTCATCGATCTGCCCGAGTACGACAAGCTGCTCGTCTGGGATAGT
D. bor     --------------------------------------------------
D. buzz ** --------------------------------------------------
D. buzz    --------------------------------------------------
D. koep    --------------------------------------------------
D. anto    --------------------------------------------------
D. gouv    --------------------------------------------------
D. serido  --------------------------------------------------
D. serie   --------------------------------------------------

                2110      2120
           ....|....|....|....|....|..
D. bor *   CTATATAAGAAGGAACACTTATTTTAG
D. bor     ---------------------------
D. buzz ** ---------------------------
D. buzz    ---------------------------
D. koep    ---------------------------
D. anto    ---------------------------
D. gouv    ---------------------------
D. serido  ---------------------------
D. serie   ---------------------------
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Guide to Authors

Drosophila Information Service prints short research, technique, and teaching articles, descriptions of new mutations, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually, with the official publication date being December. The annual issue will include material submitted during the calendar year. To help us meet this target date, we request that submissions be sent by 15 December, but articles are accepted at any time. A receipt deadline of 31 December is a firm deadline, due to printer submission schedules. Electronic submissions are encouraged, and may be required for lengthy or complex articles.

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Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Jin and Brown, 1990; Waters *et al*., 1990). At the end of the article, references should be listed **alphabetically** by senior author, listing all authors with initials, date, journal, volume and page numbers. Titles will not be included except for books, unpublished theses, and articles in press. An example format is:

Green, R.L., 1998, Heredity 121: 430-442.

Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Note the initials are before each name except for the senior author.