COLOGNE GRADUATE SCHOOL OF AGEING RESEARCH

Characterising antibody immunity and ageing in a short-lived teleost

William John Bradshaw

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Antibodies

B-cells

Ageing has major effects on B-cell immunity

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- Reduced naïve B-cell output
- Fewer unique antibody sequences
- Decreased responsiveness to vaccination
- Impaired antibody quality
- ...

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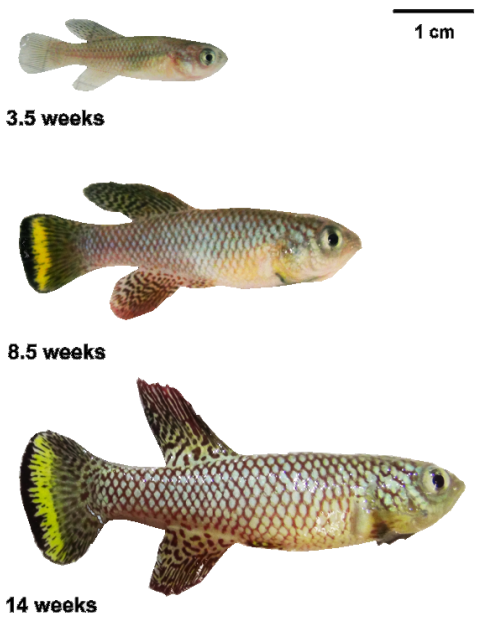
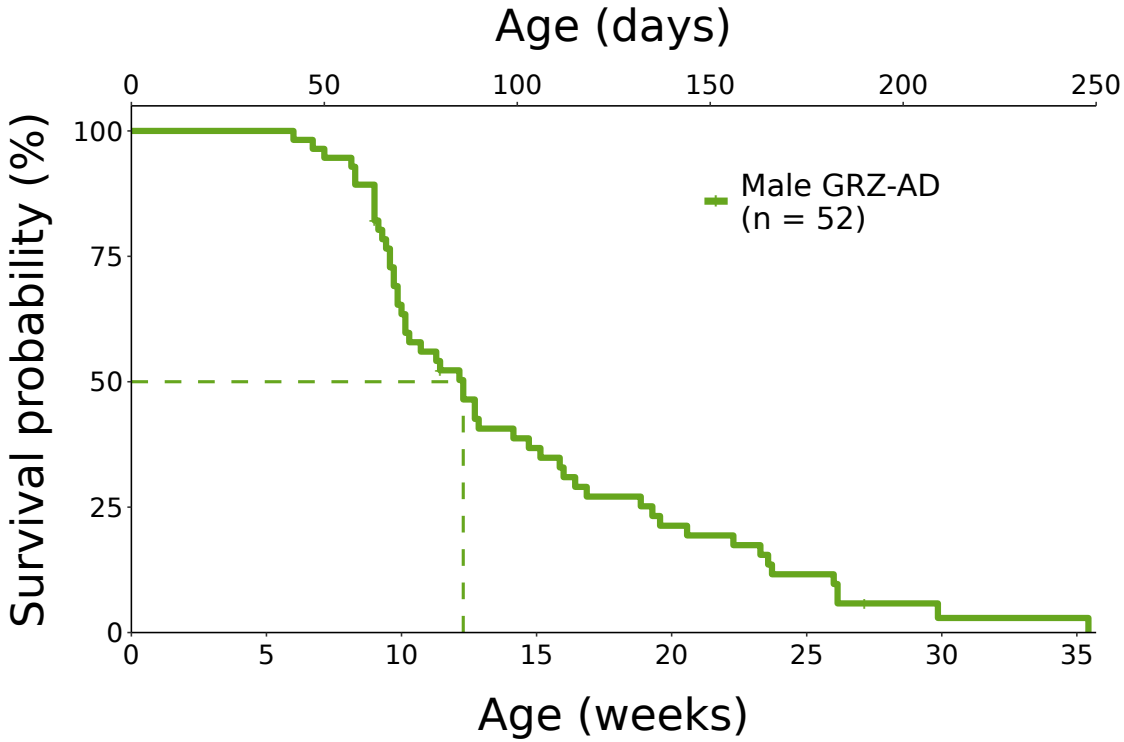
- Very little known outside humans and mice
- Almost all data comes from peripheral blood
- No spatial resolution (different organs)

The turquoise killifish as a model for antibody ageing

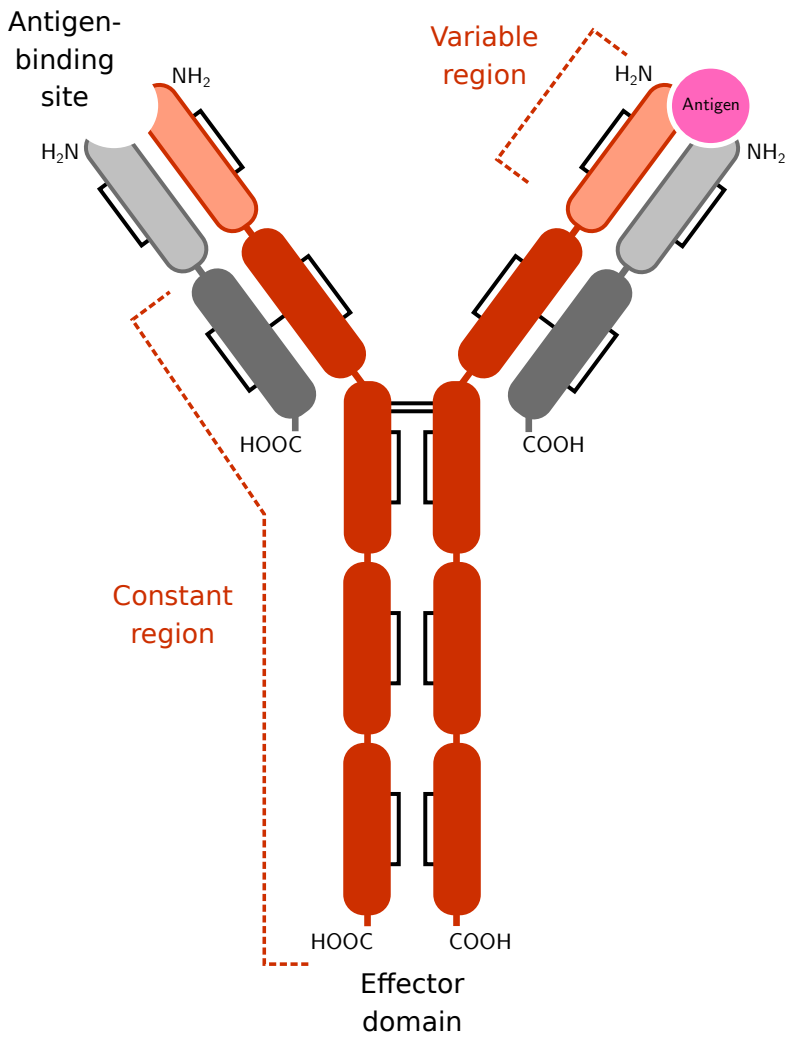


Yumi Kim



How does B-cell immunity change with age in killifish?

How does the **antibody heavy-chain repertoire** change with age in killifish?



How does the **antibody heavy-chain repertoire** change with age in killifish?

Characterise IGH locus

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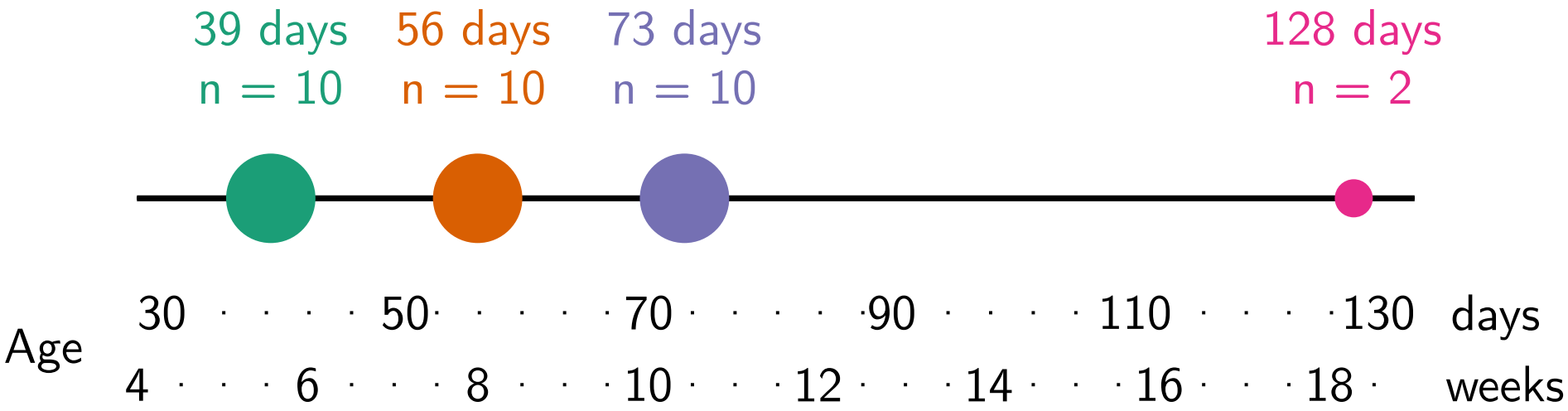
Characterise IGH locus → **Design IgSeq protocol**

How does the **antibody heavy-chain repertoire** change with age in killifish?

Characterise IGH locus → **Design IgSeq protocol** → **Investigate repertoire ageing**

Sample design – killifish ageing study



GRZ ♂

Clonal antibody diversity

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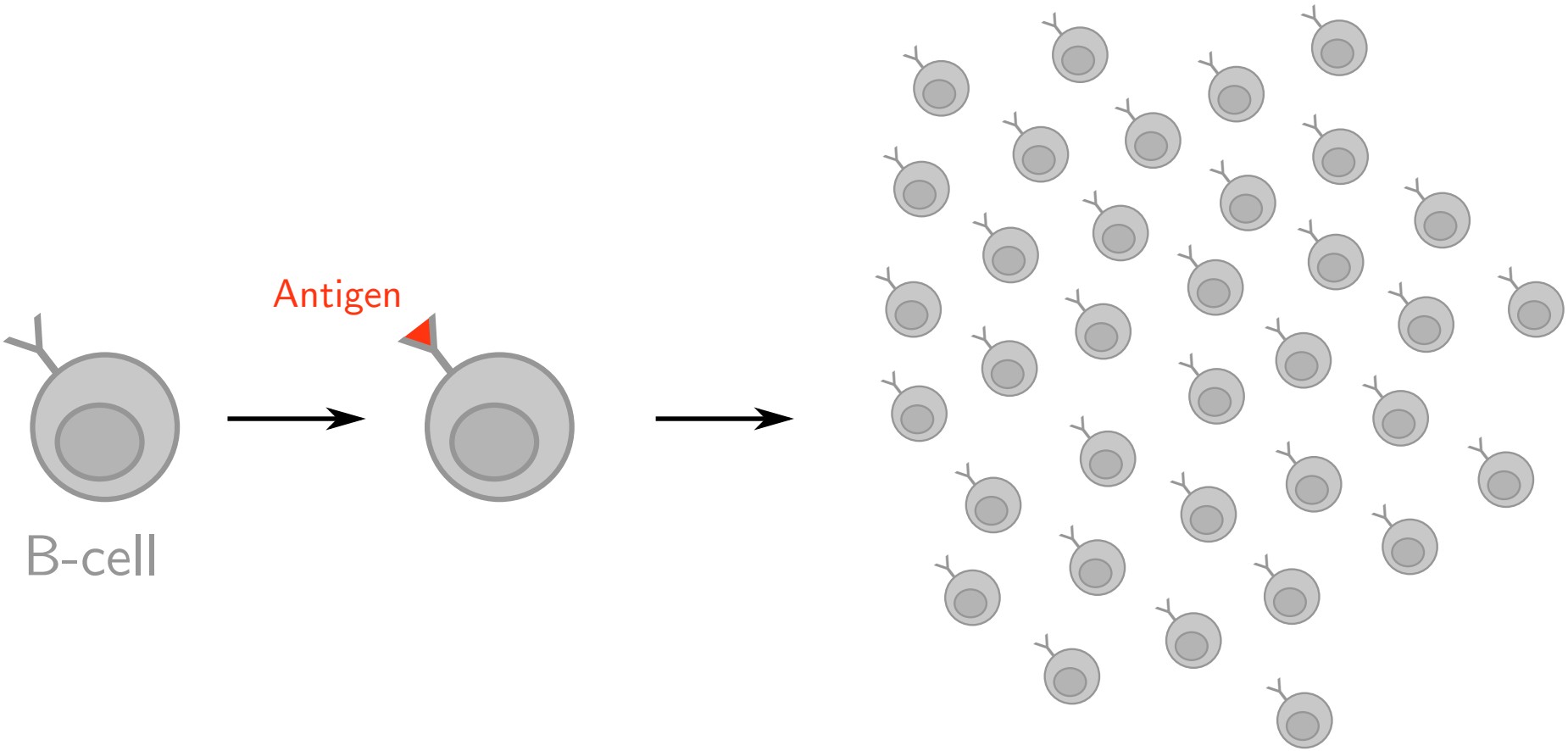
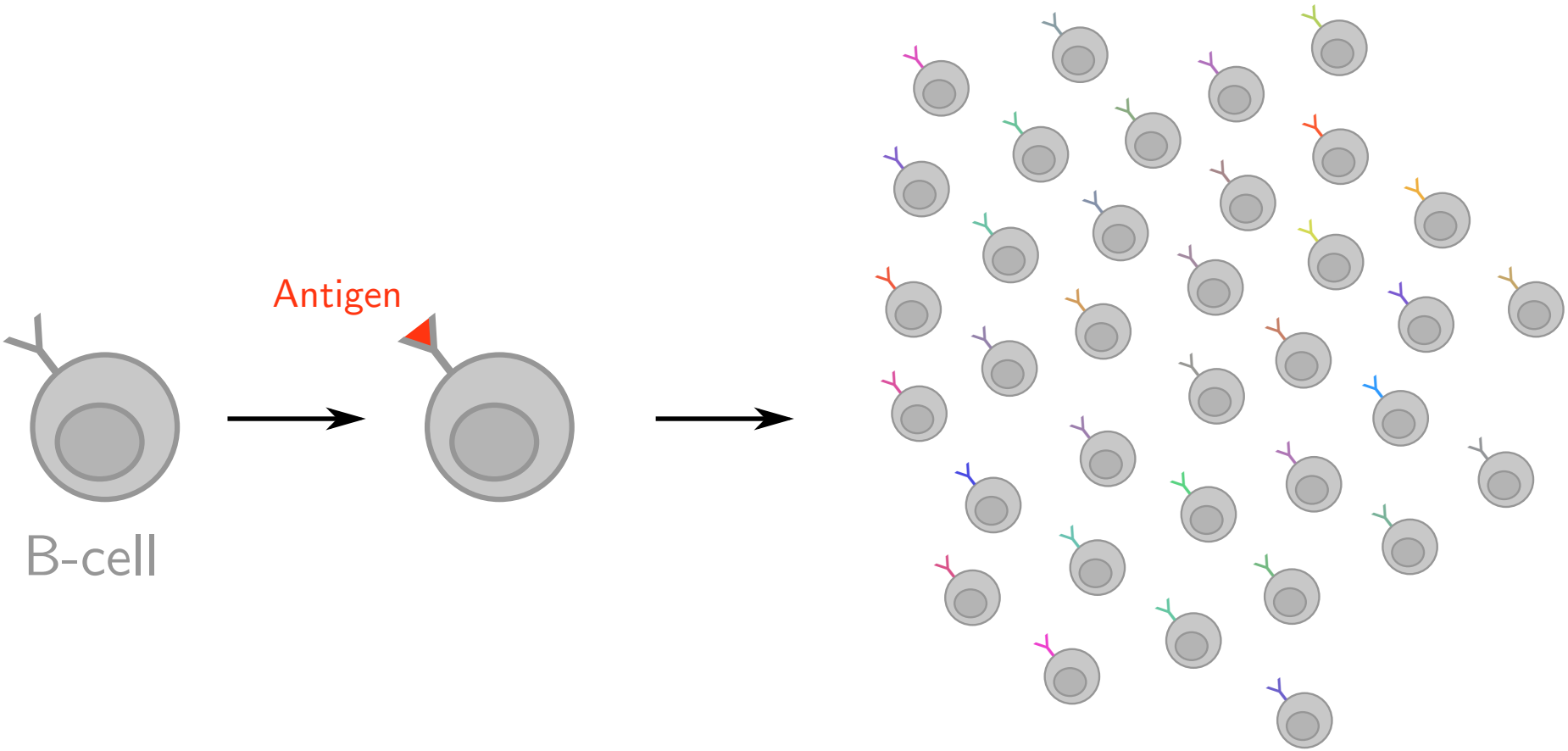
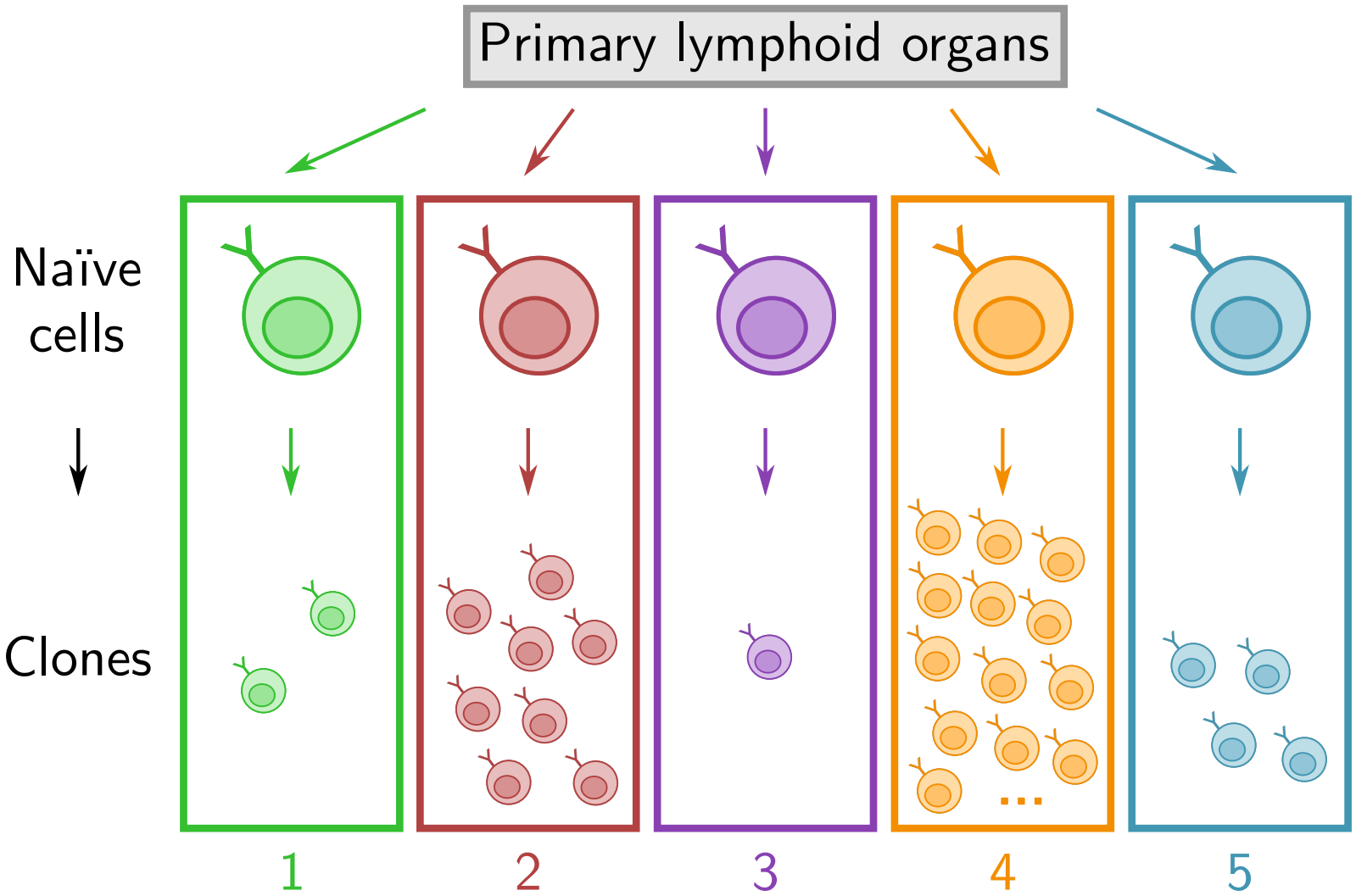


Clonal antibody diversity

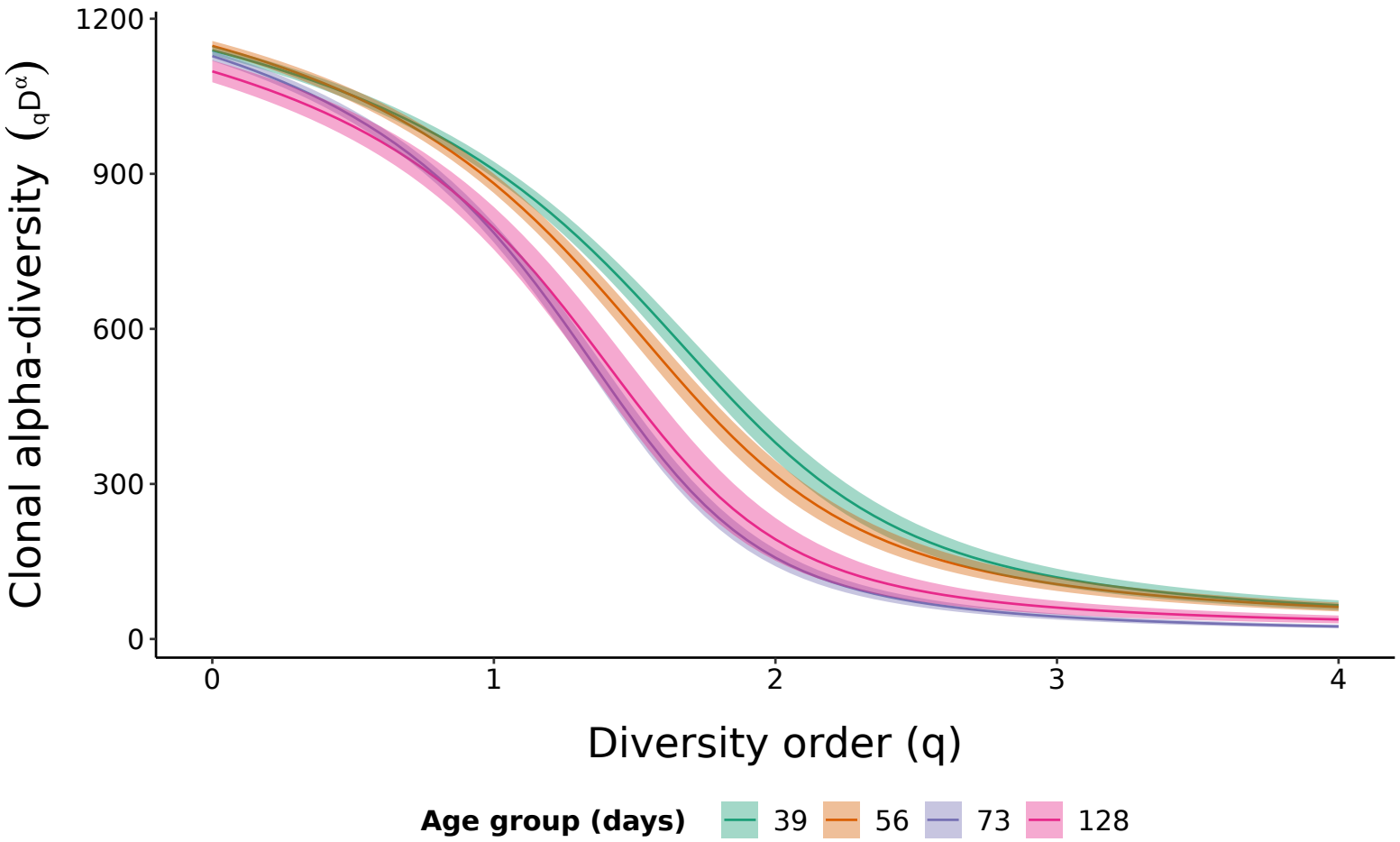


Clonal antibody diversity



Clonal alpha diversity in the killifish antibody repertoire

Clonal alpha diversity in the killifish antibody repertoire declines with age



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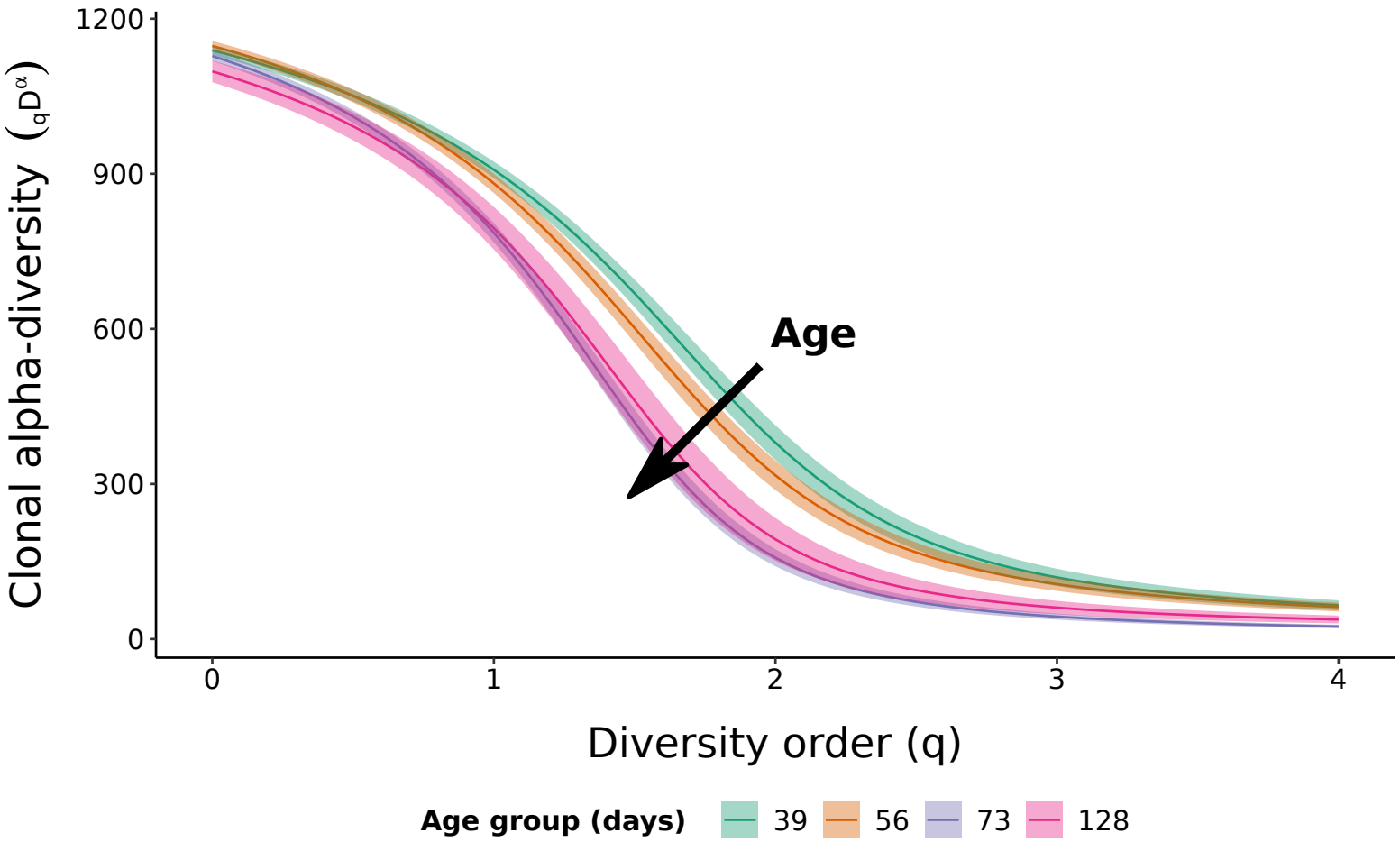

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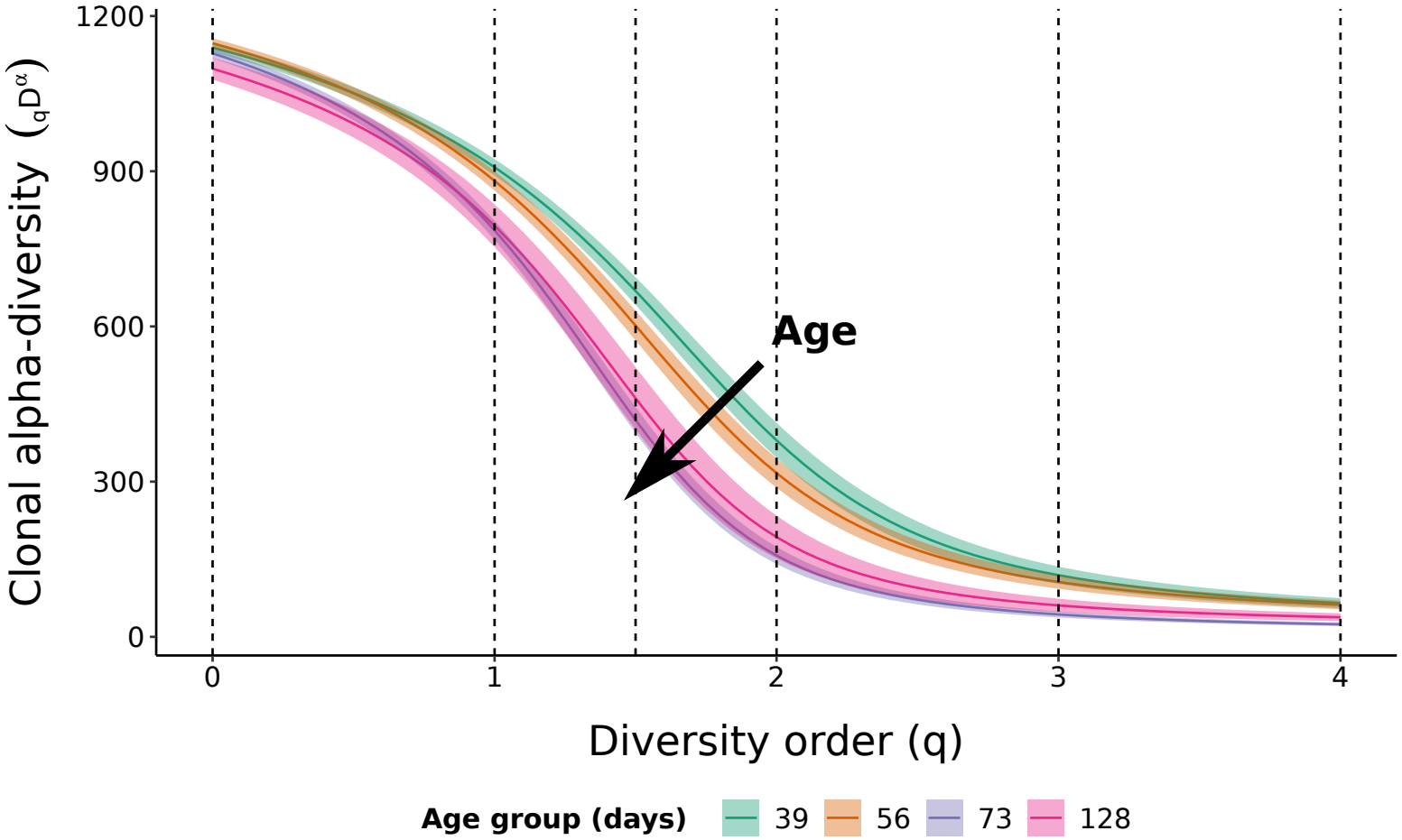
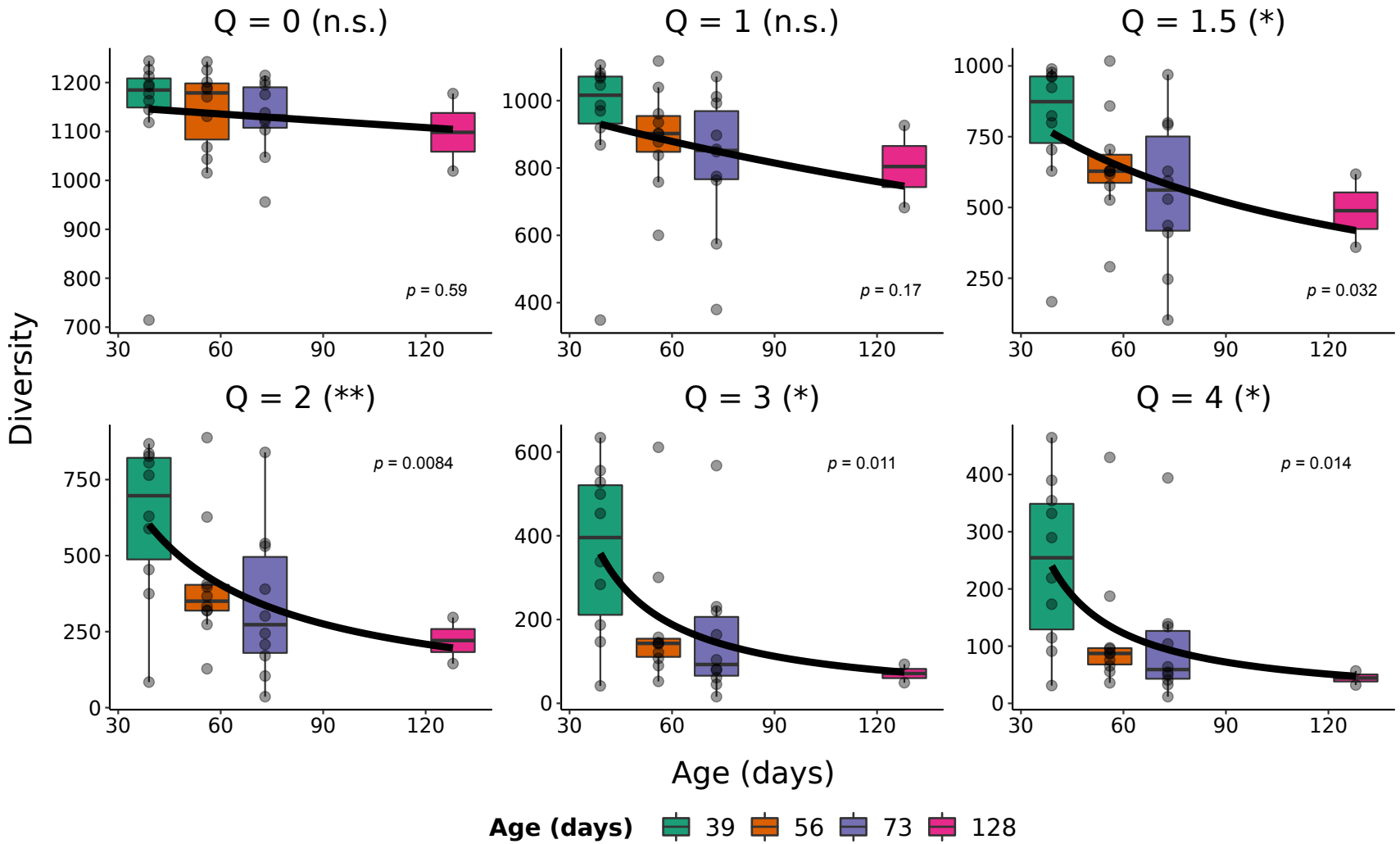
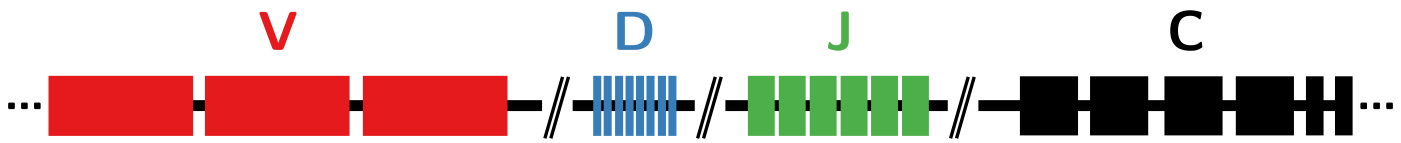


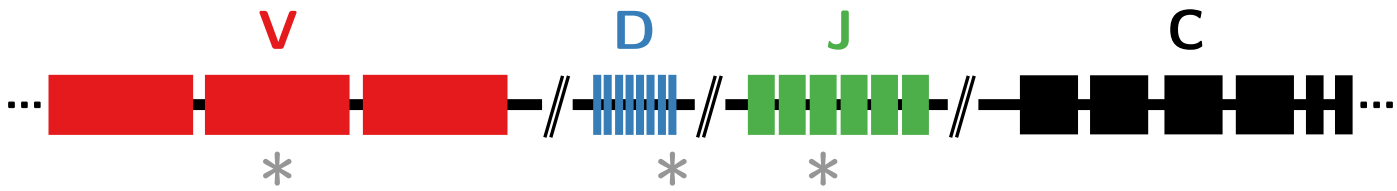
Clonal alpha diversity in the killifish antibody repertoire declines with age at high diversity orders



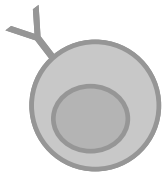
VJ alpha diversity in the killifish antibody repertoire



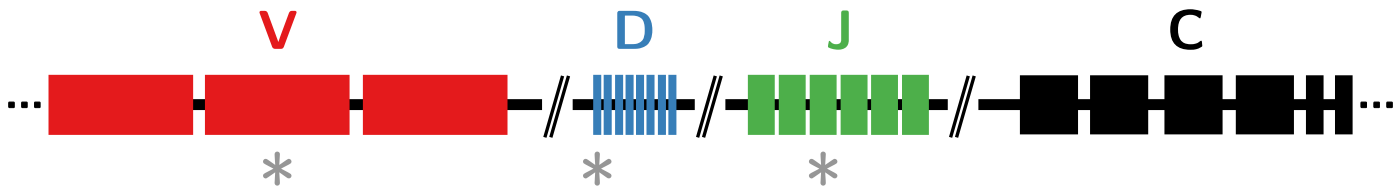
VJ alpha diversity in the killifish antibody repertoire

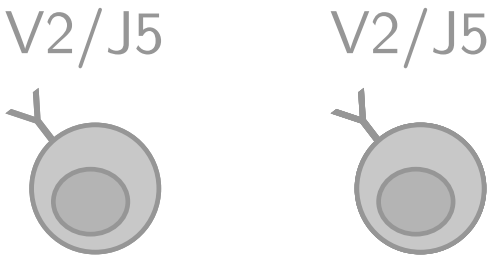


V2/J5

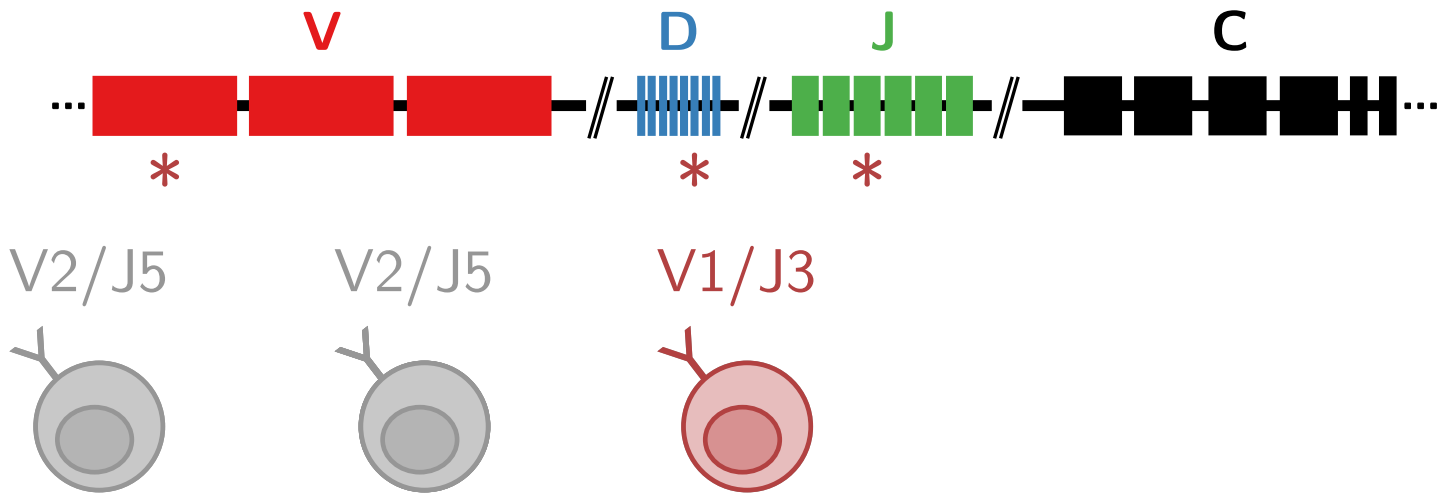


VJ alpha diversity in the killifish antibody repertoire

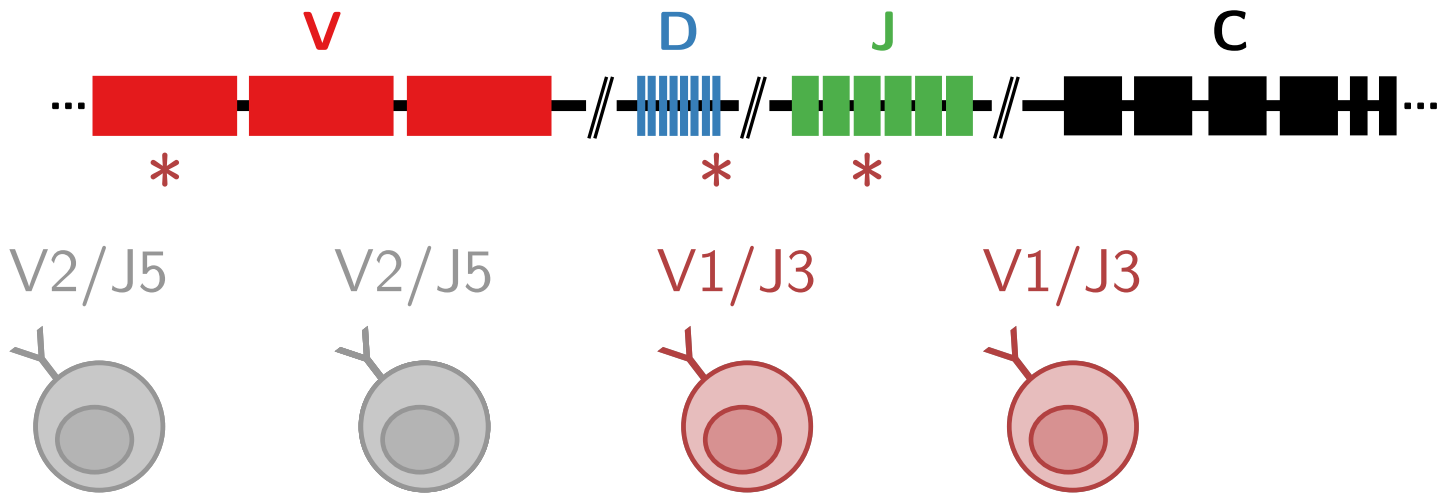




VJ alpha diversity in the killifish antibody repertoire



VJ alpha diversity in the killifish antibody repertoire



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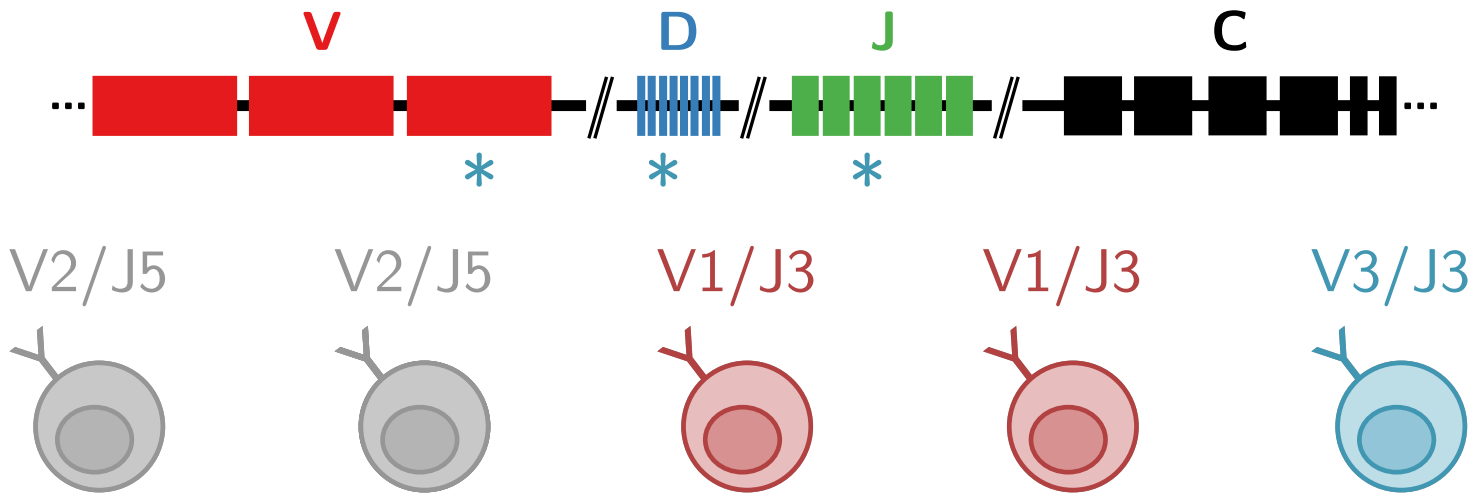

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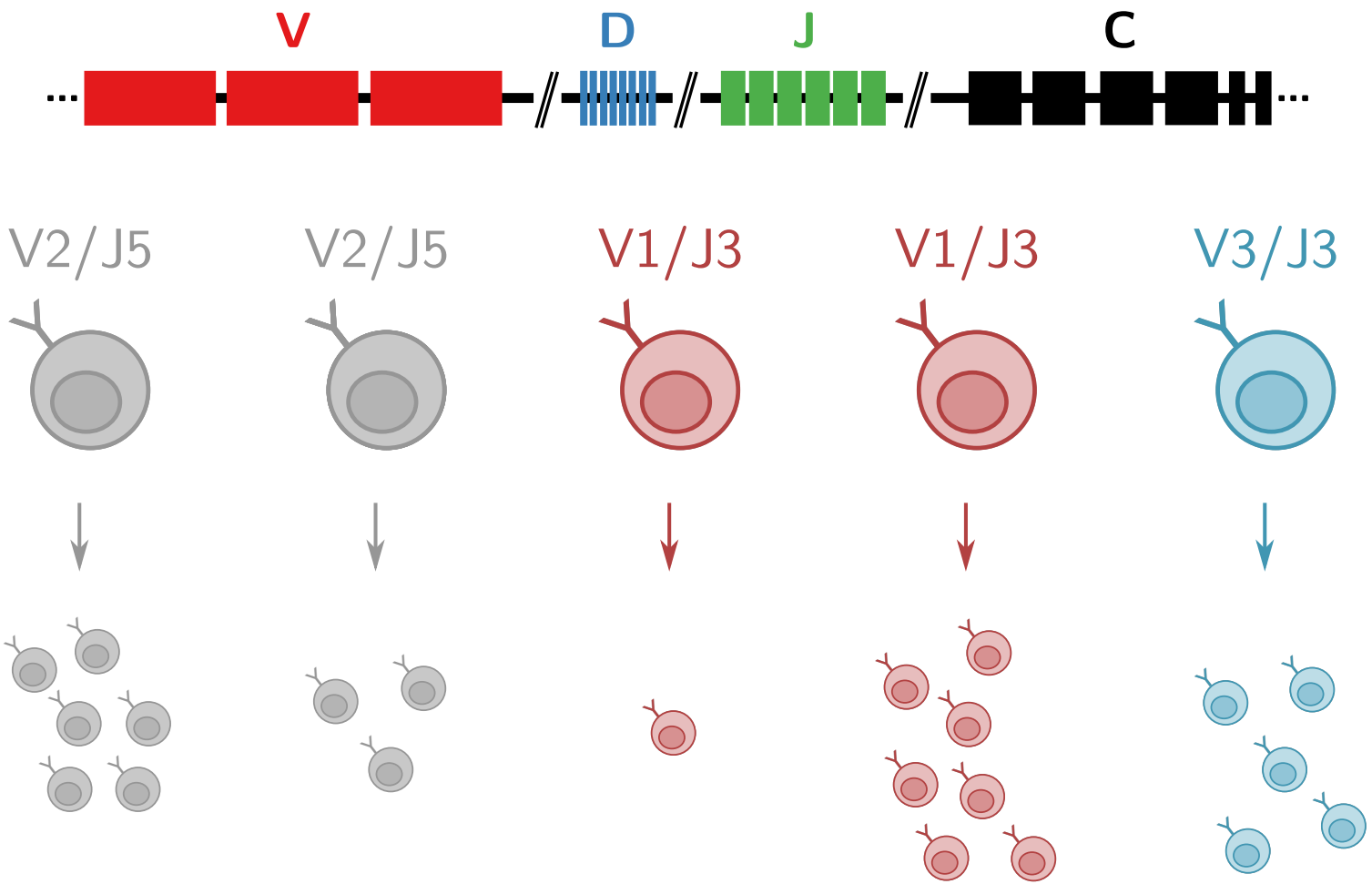
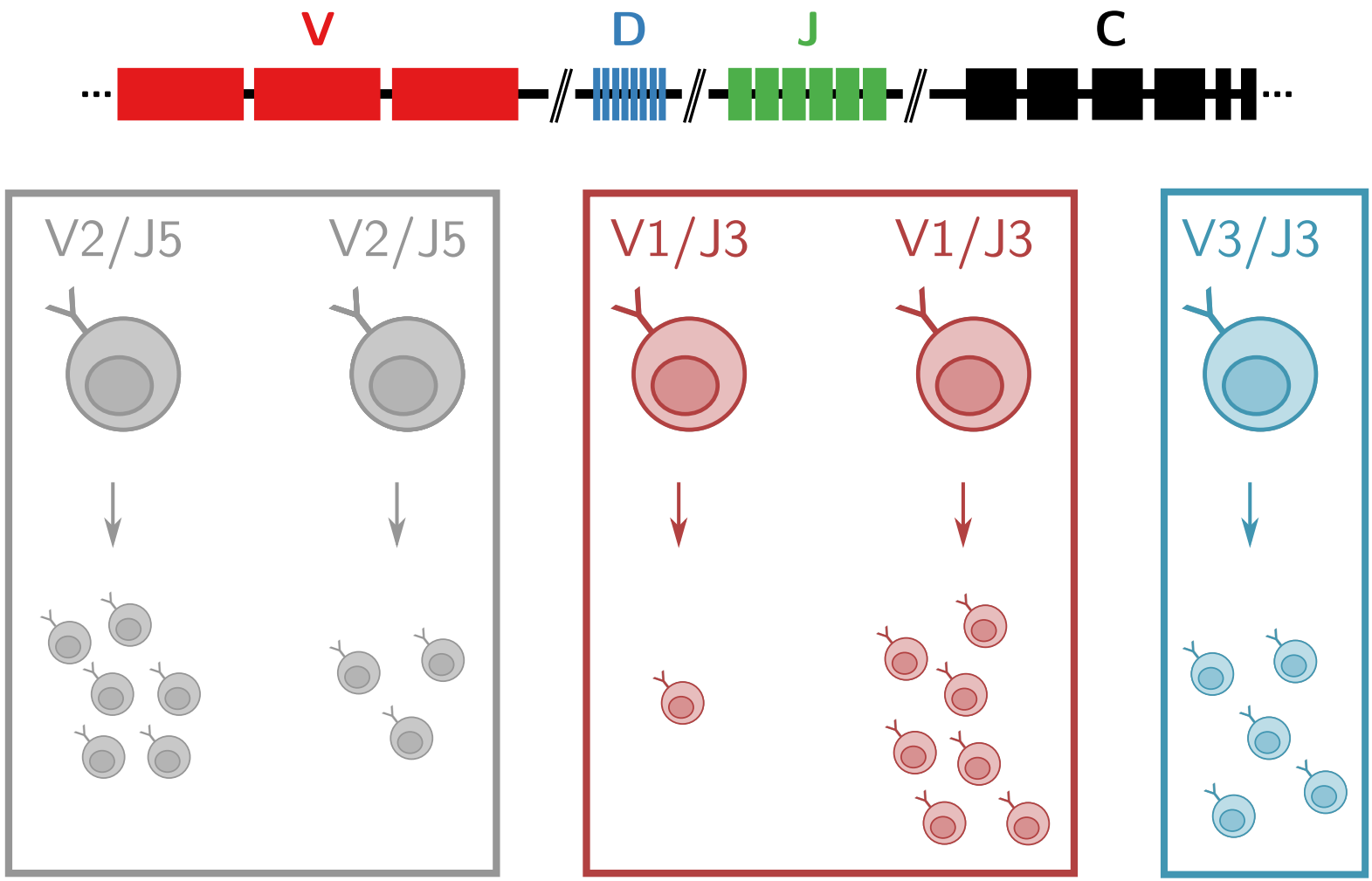
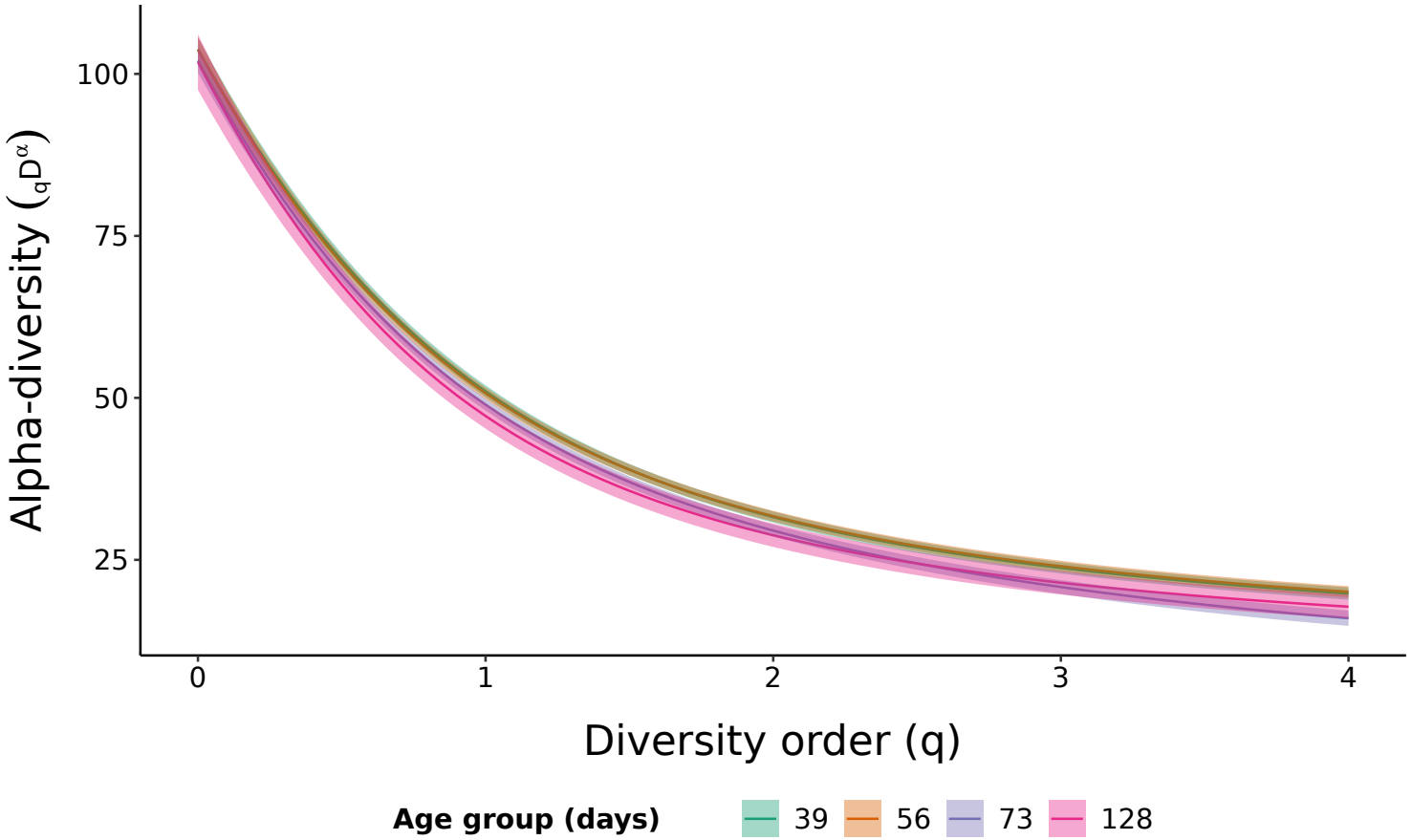


VJ alpha diversity in the killifish antibody repertoire



VJ alpha diversity in the killifish antibody repertoire

VJ alpha diversity in the killifish antibody repertoire **does not decline** with age



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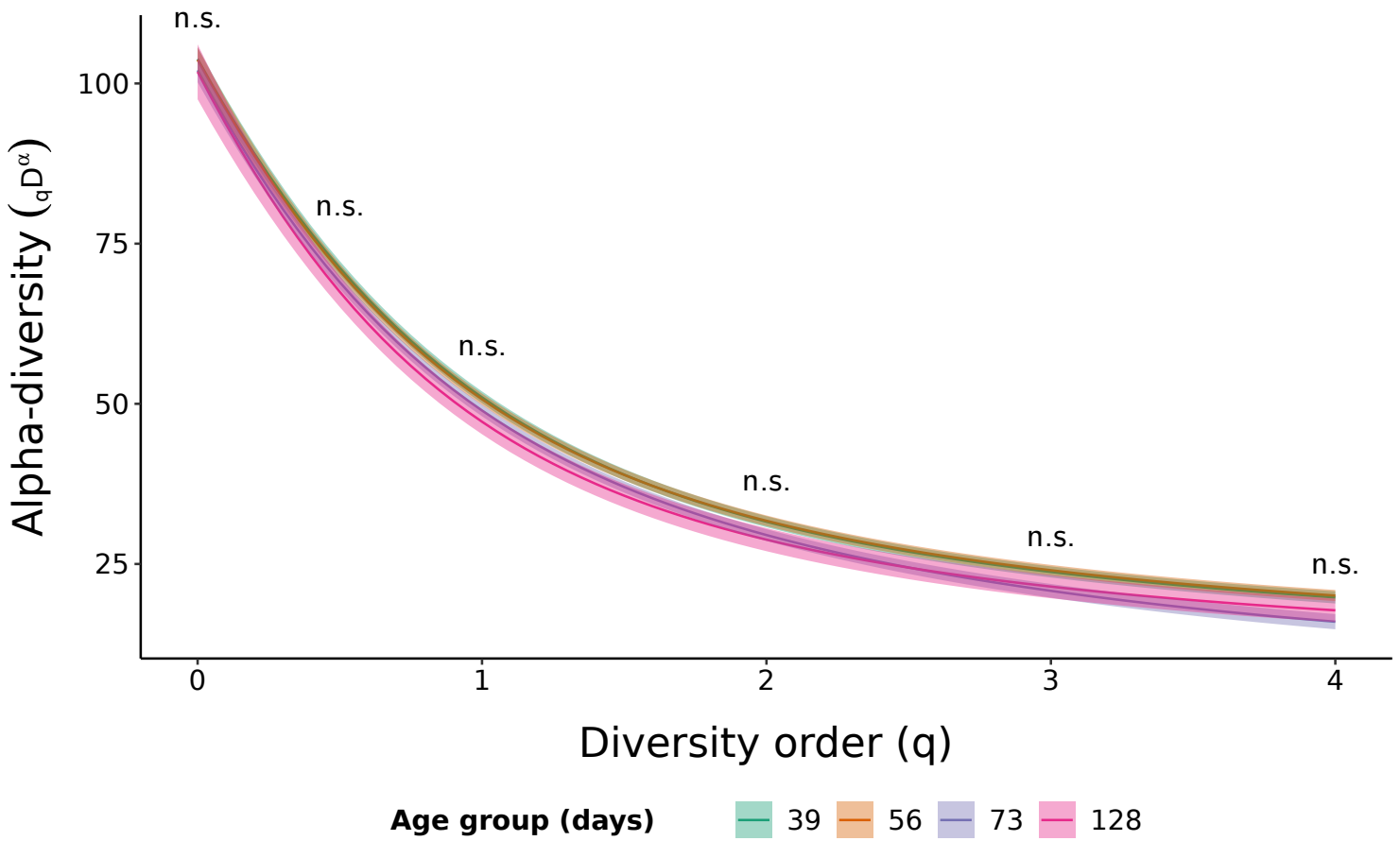
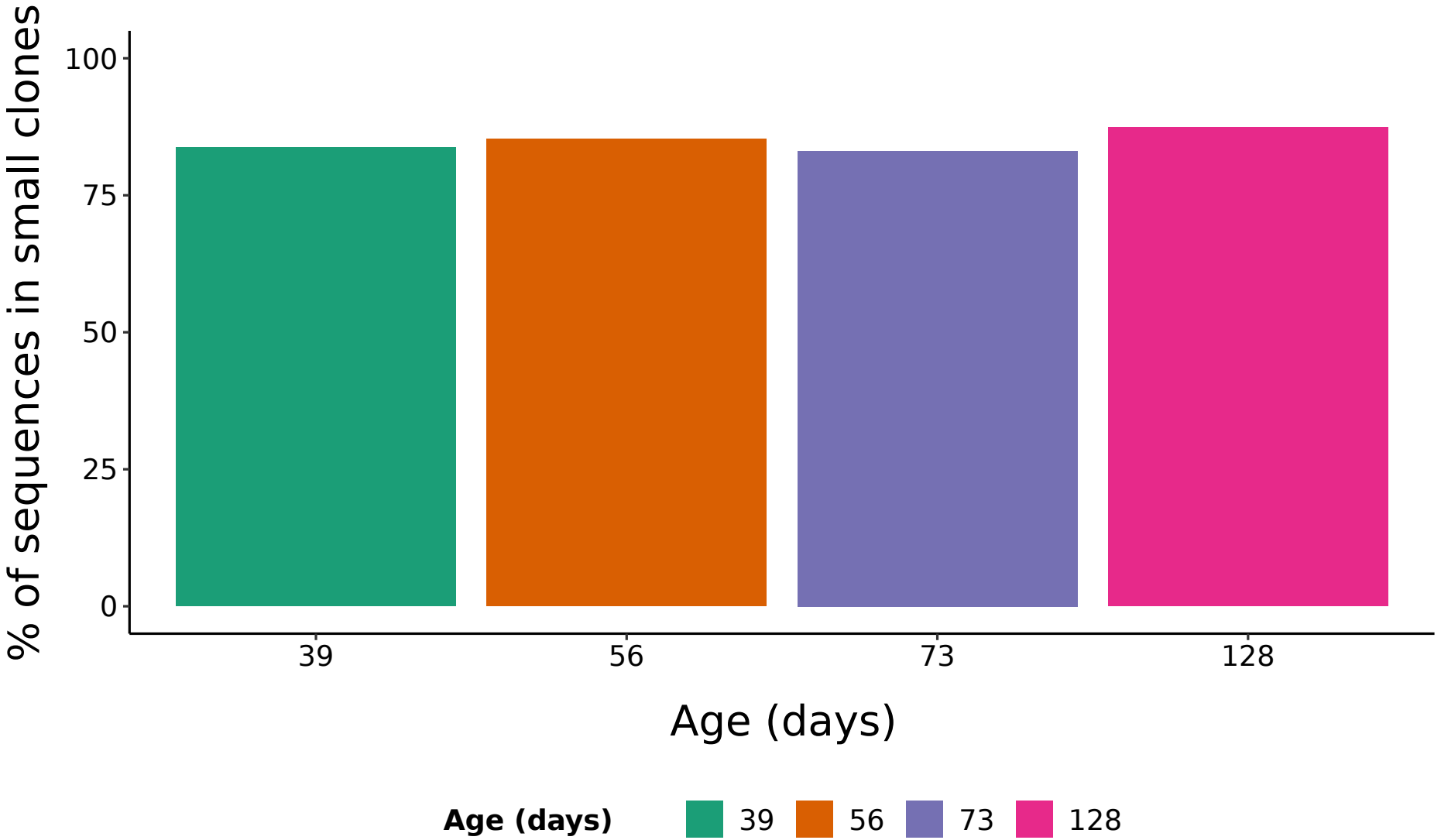
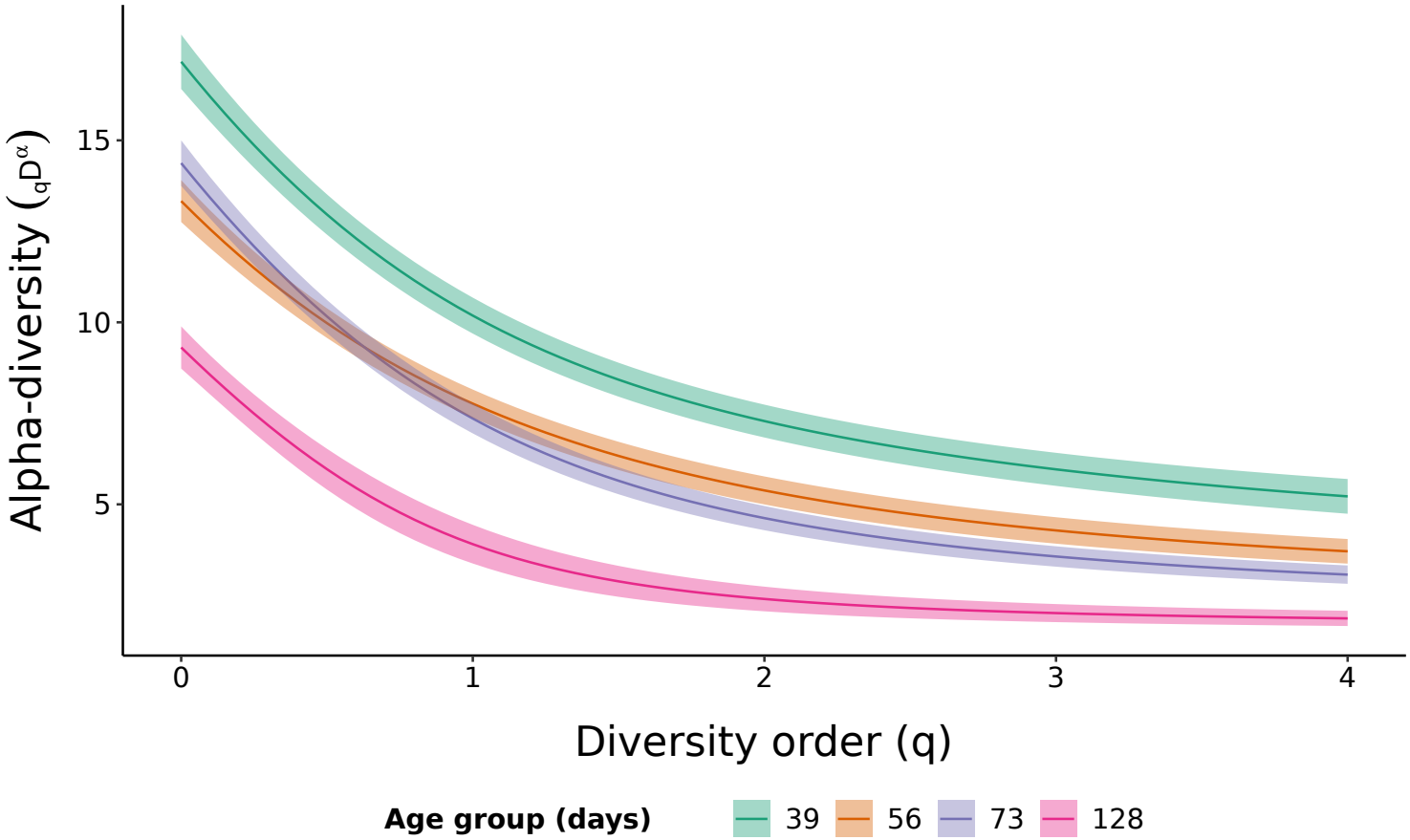


Most sequences in killifish repertoires are from small clones

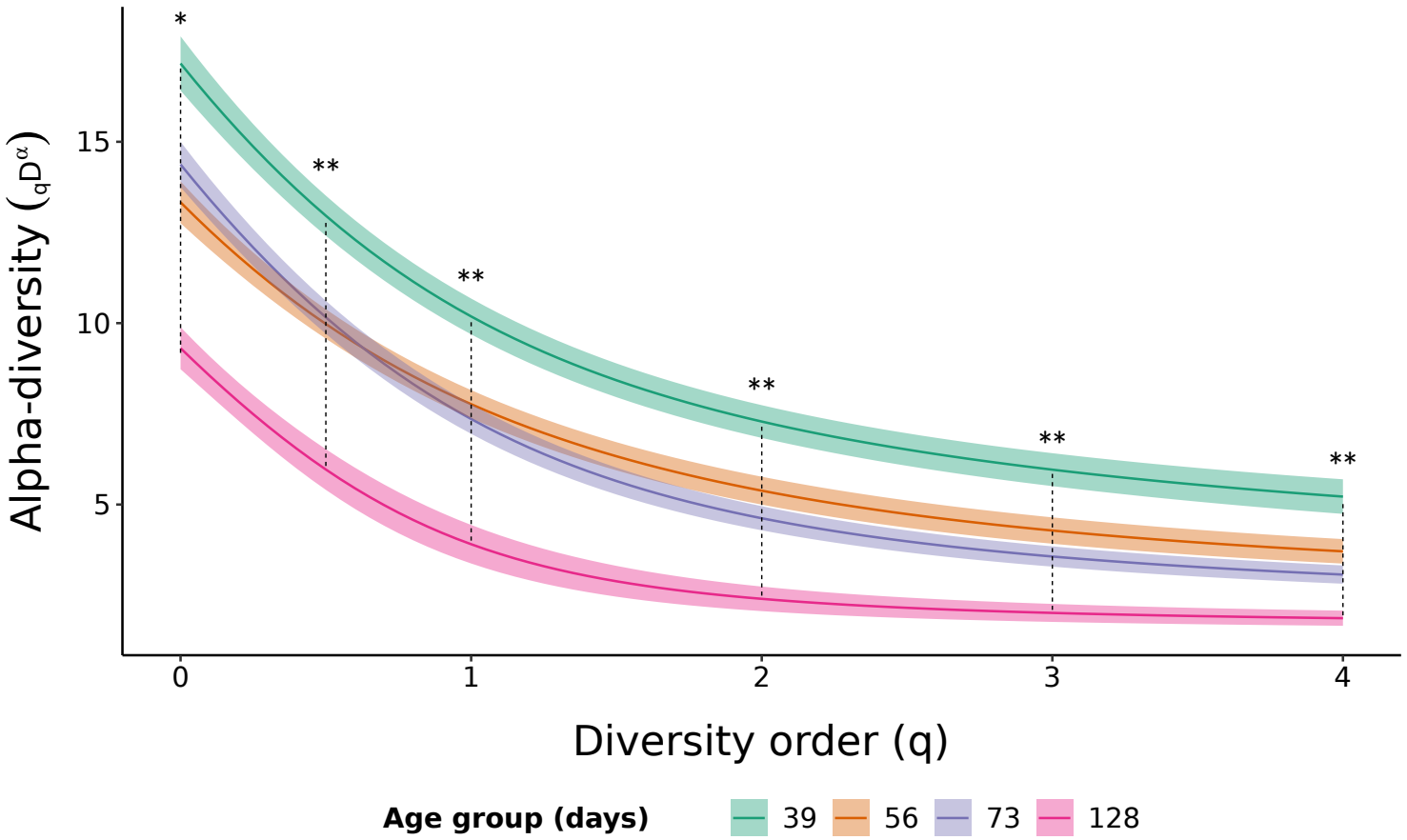


VJ alpha diversity of **large clones**

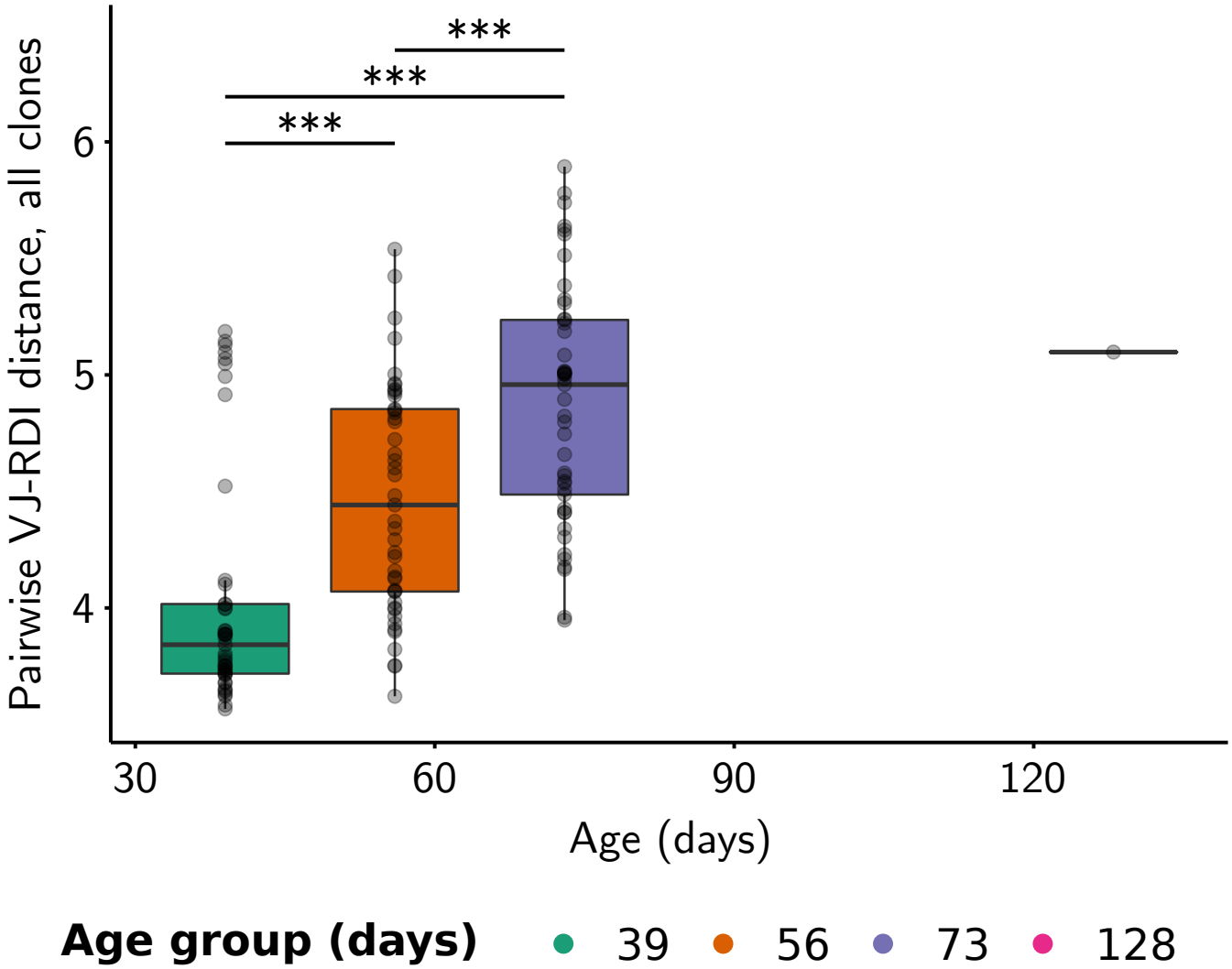
VJ alpha diversity of **large clones does decline** with age



VJ alpha diversity of **large clones does decline** with age



Killifish VJ repertoires become **more dissimilar** with age



Summary I

Age

Antibody repertoire diversity

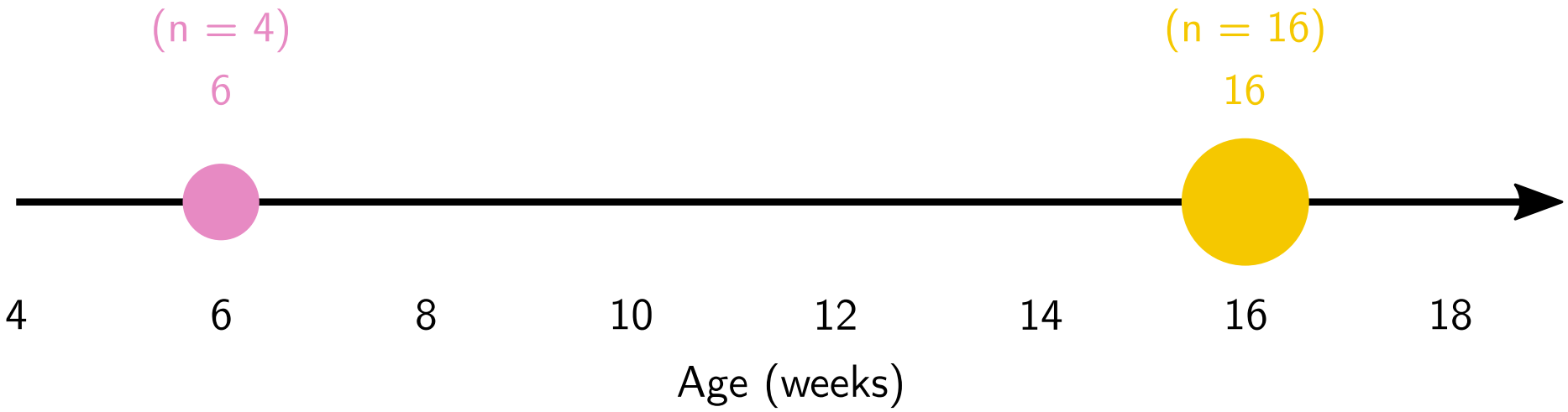
Small (naïve) clones

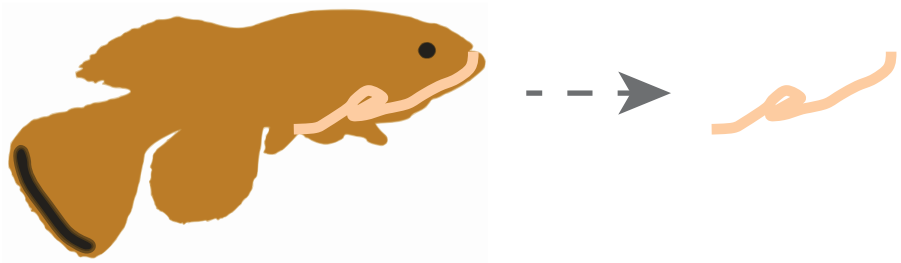
Large (expanded) clones

Alpha diversity

Beta diversity

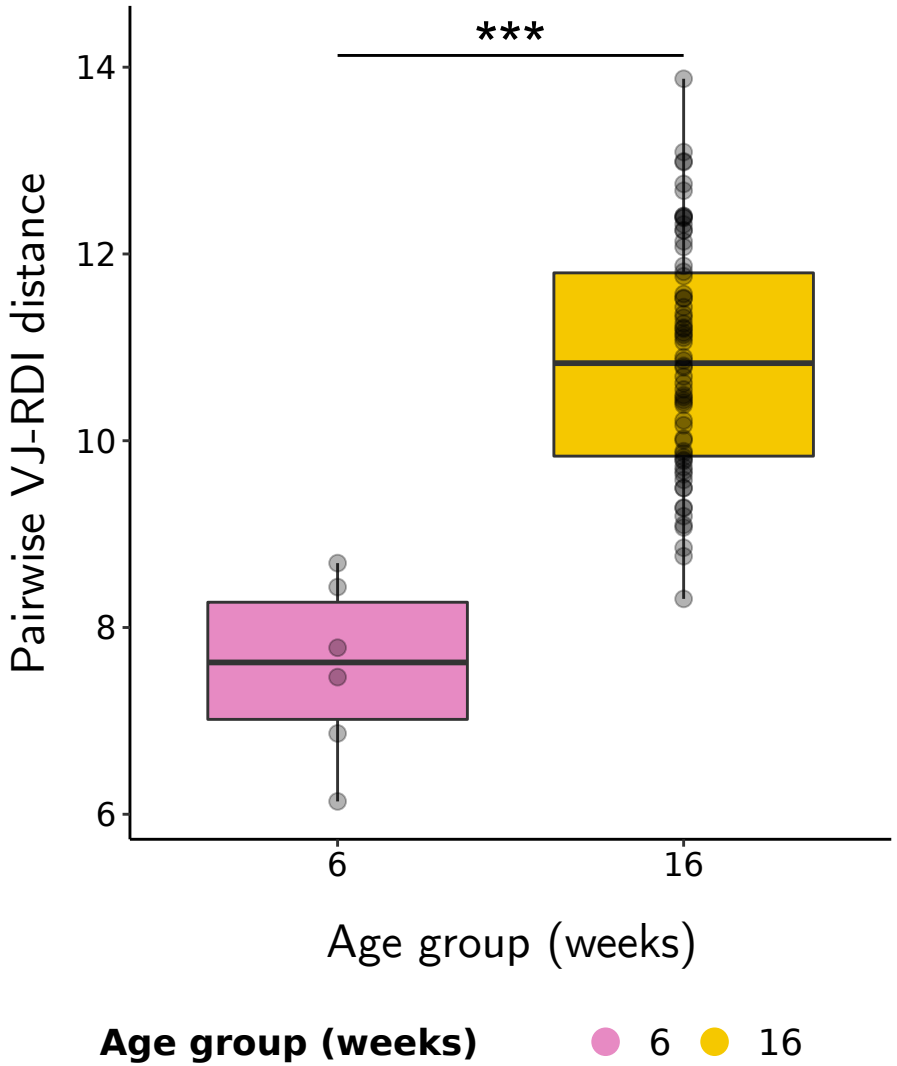
Sample design – gut repertoire study



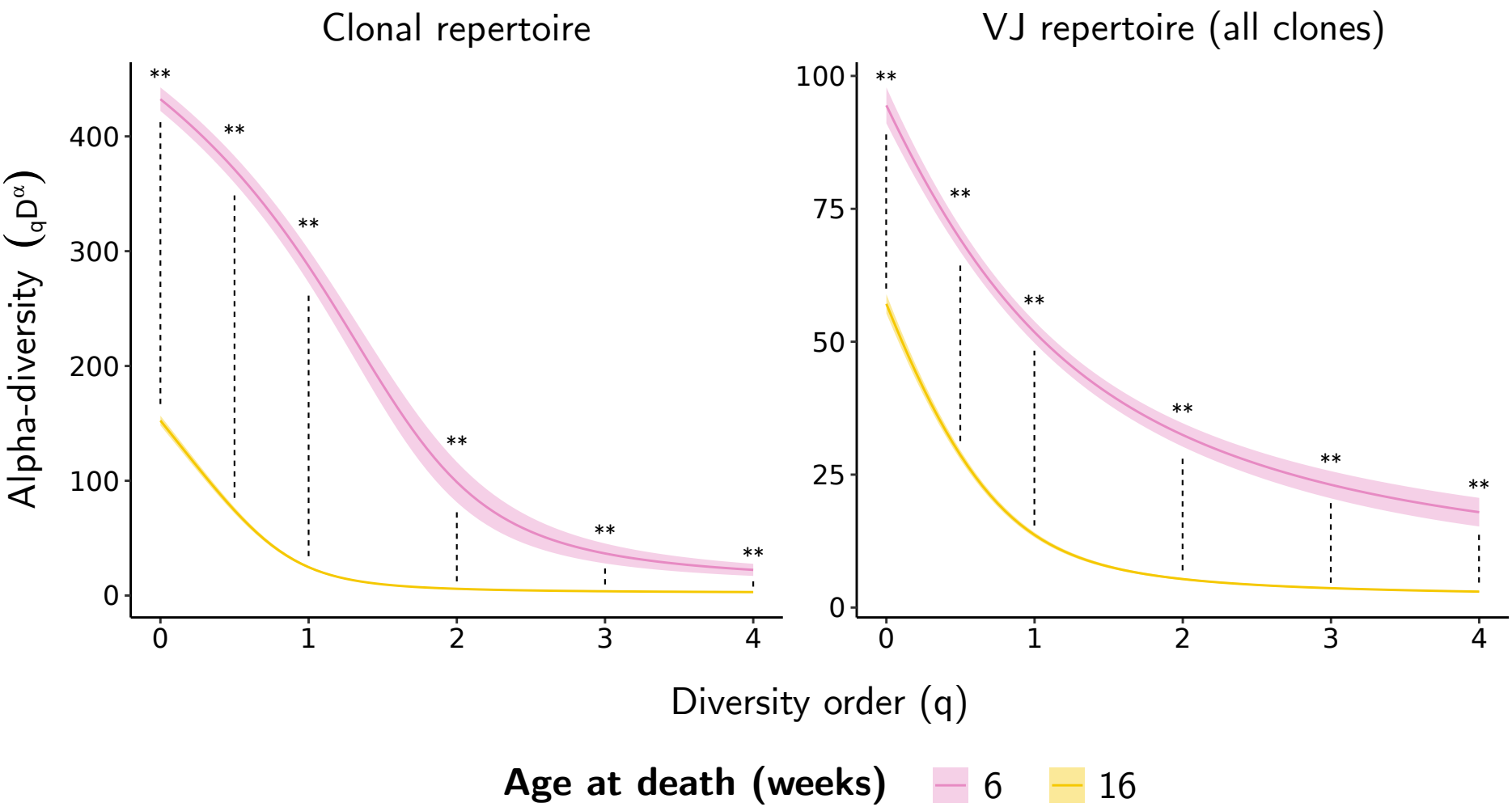


Samples collected for Smith et al., eLife 2017

Killifish gut repertoires become much more dissimilar with age



Gut repertoire alpha diversity declines dramatically with age in turquoise killifish



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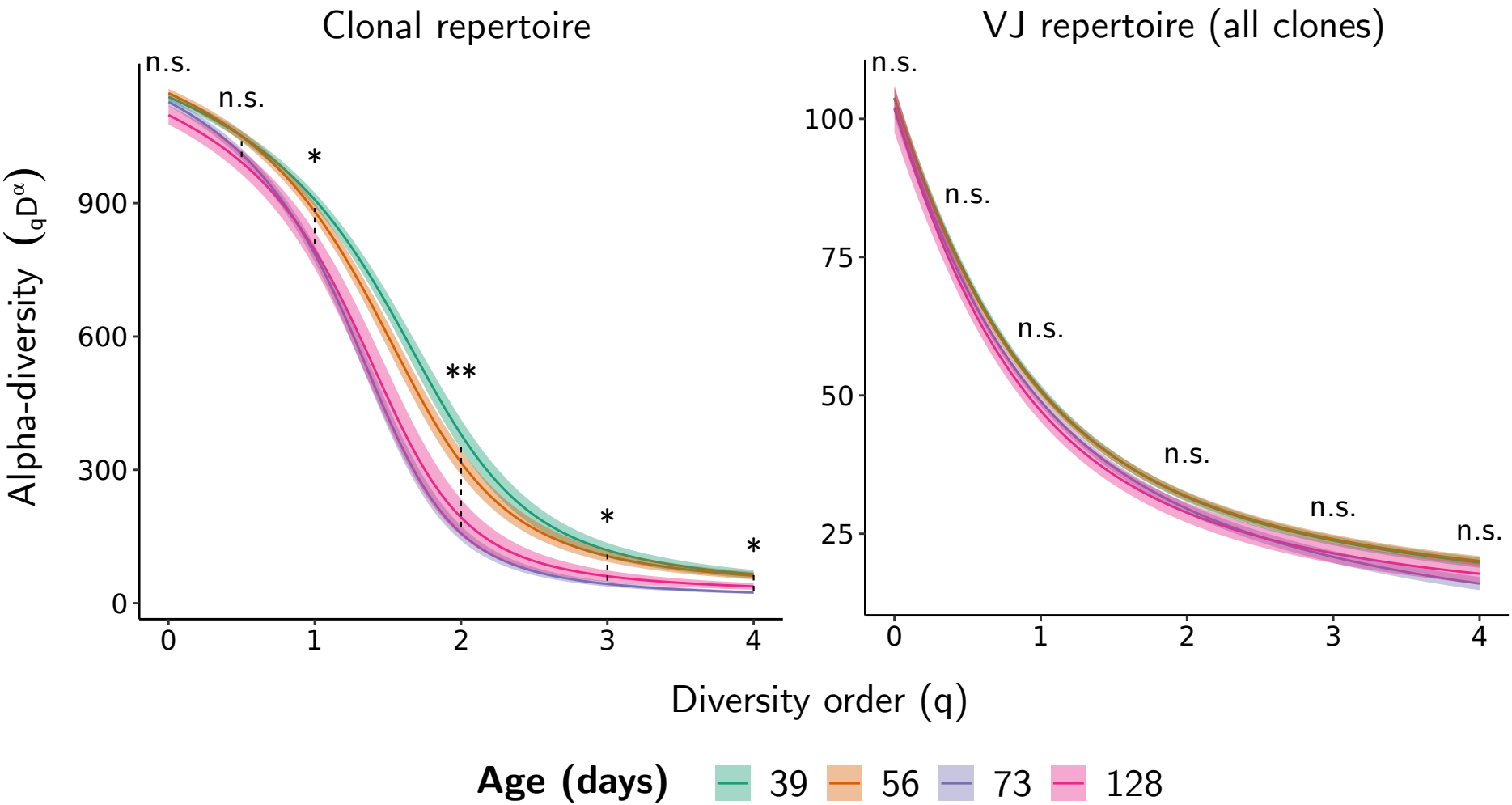

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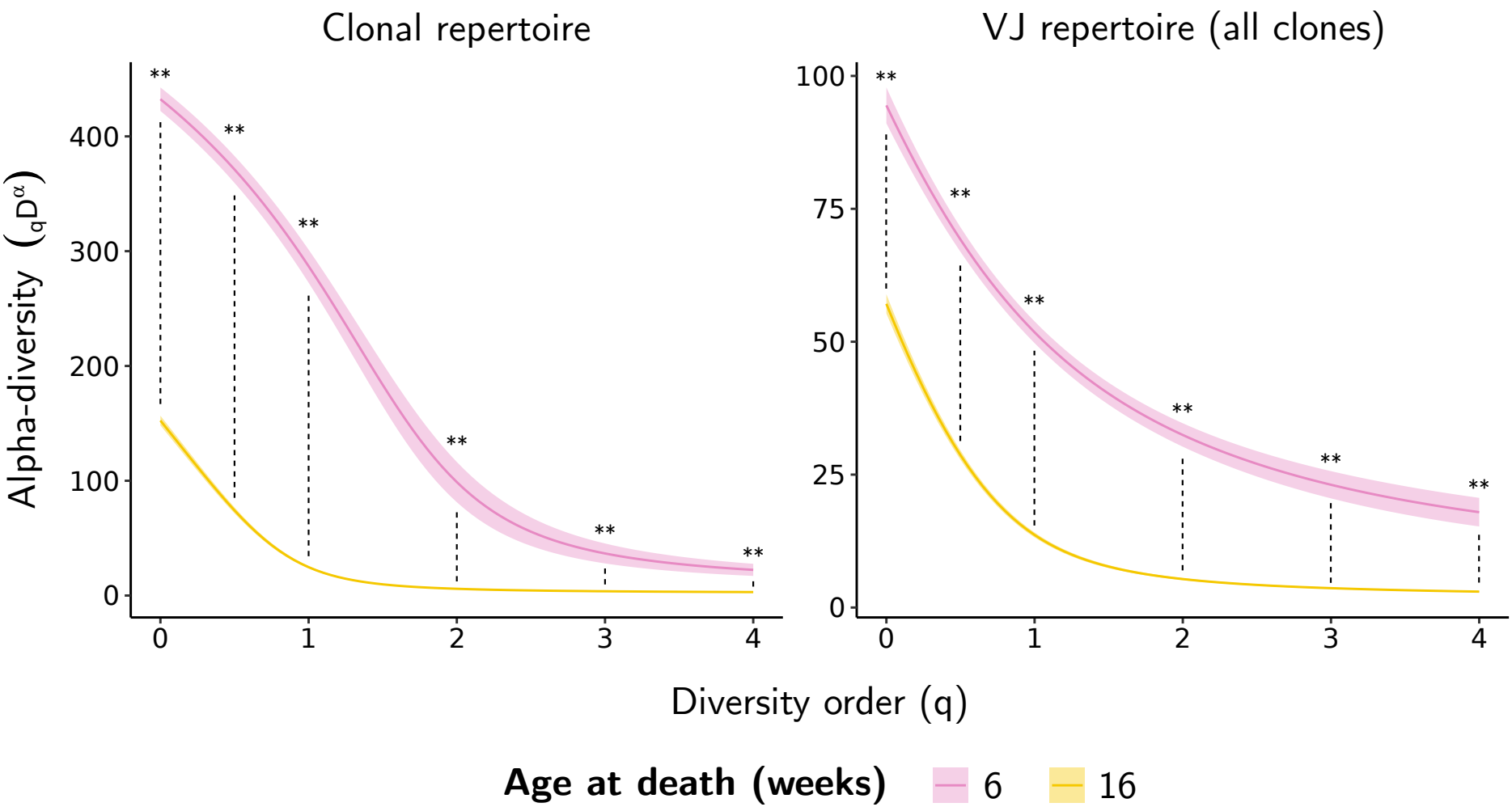

Why the difference?

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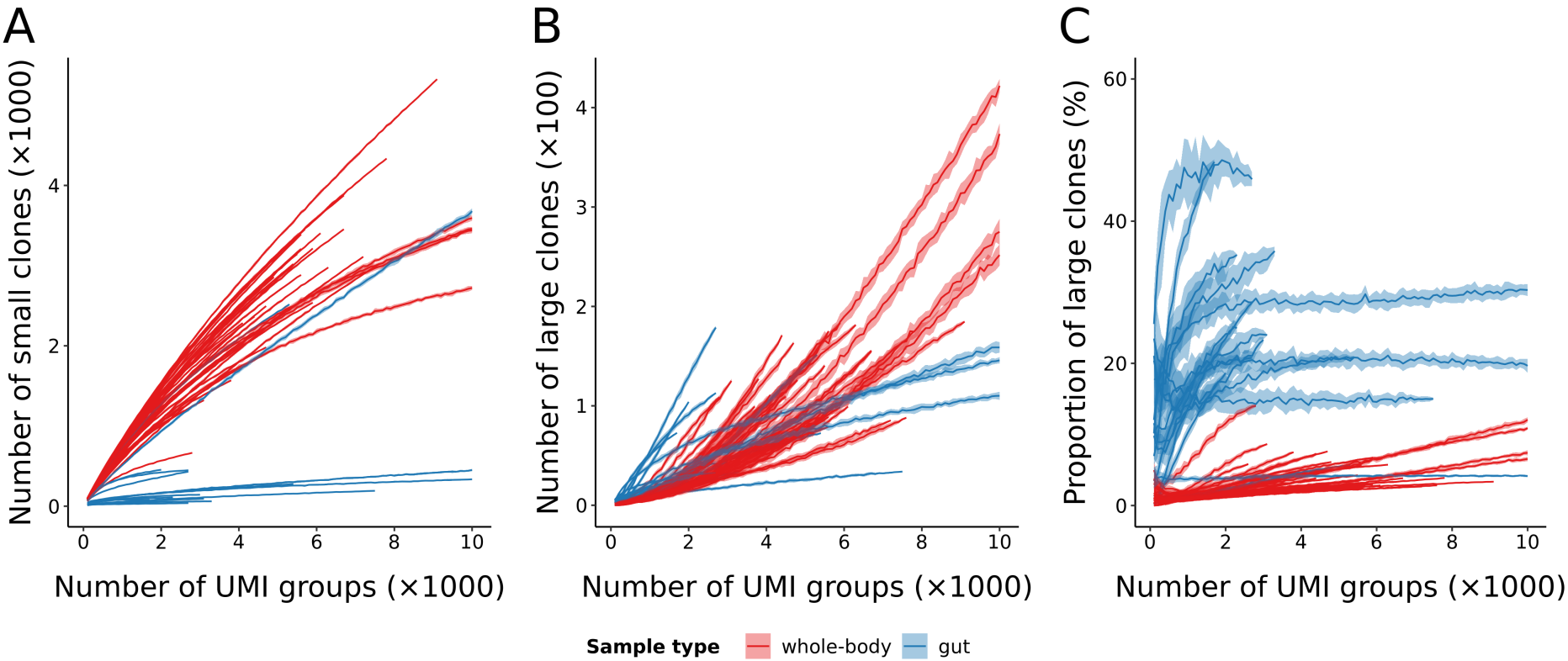
1. Difference in **environment**?
   - Intense antigen exposure from gut microbiota
   - Greater antigen exposure → more clonal expansion, less diversity
2. Difference in **clonal composition**?
   - Whole body includes primary lymphoid organs, intestine does not
   - → More small naïve clones in whole-body samples than gut samples
   - Larger clones more age-sensitive → stronger age effect in gut samples

Killifish gut repertoires contain fewer **small** clones than whole-body repertoires



Summary II

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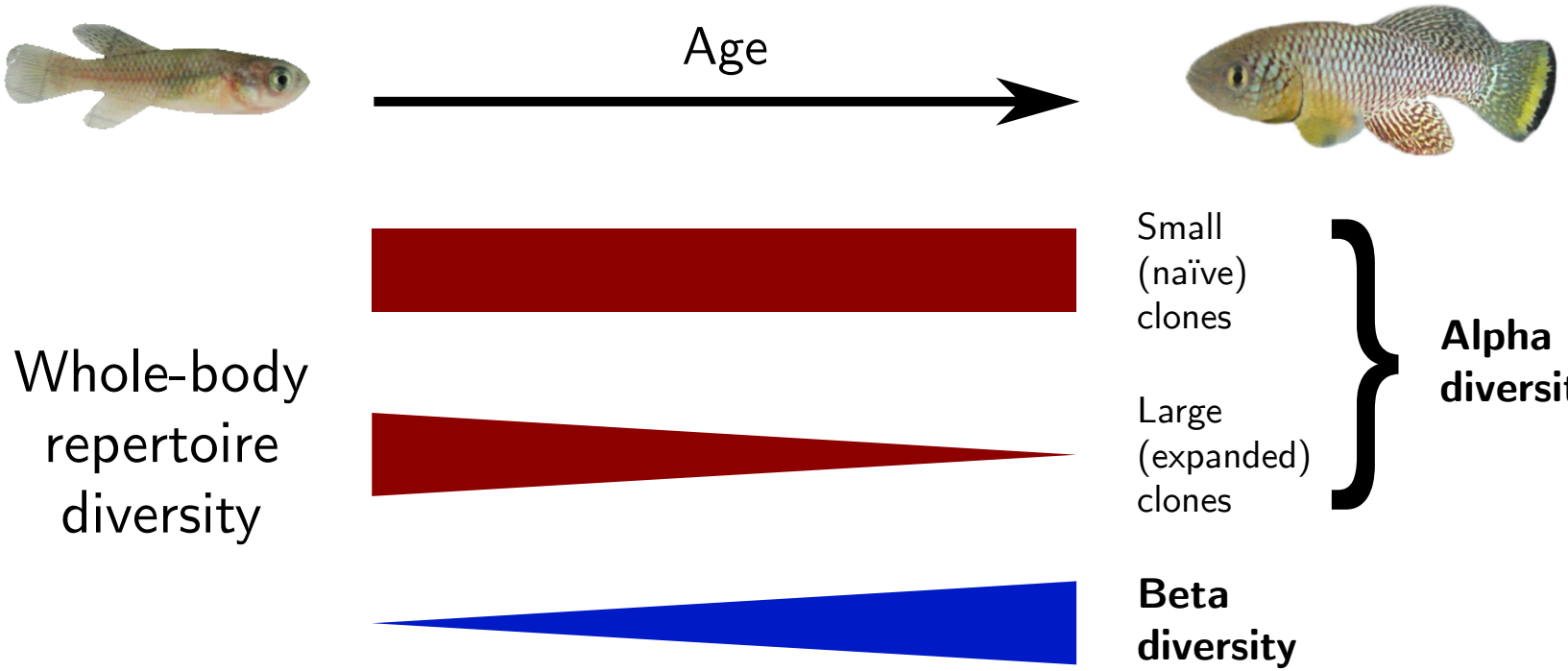
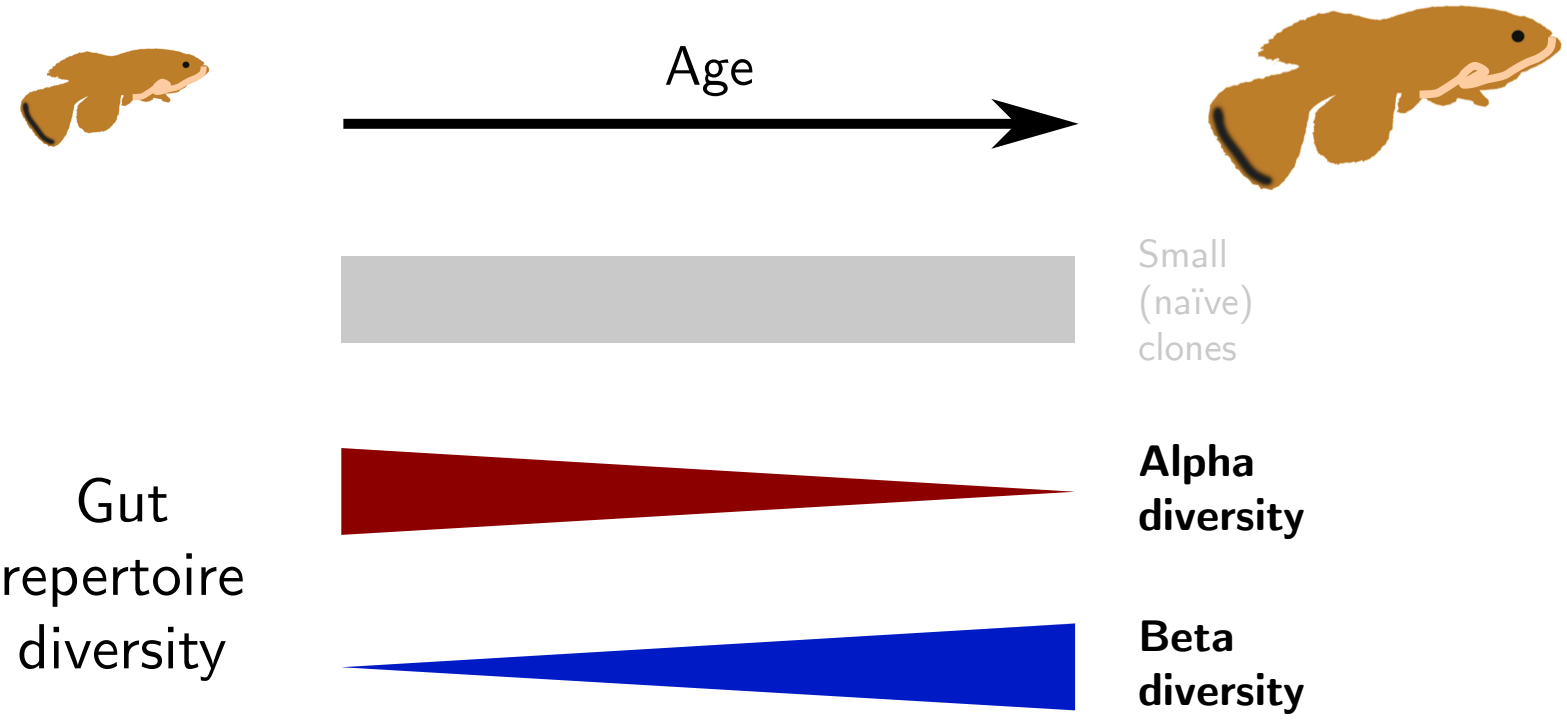
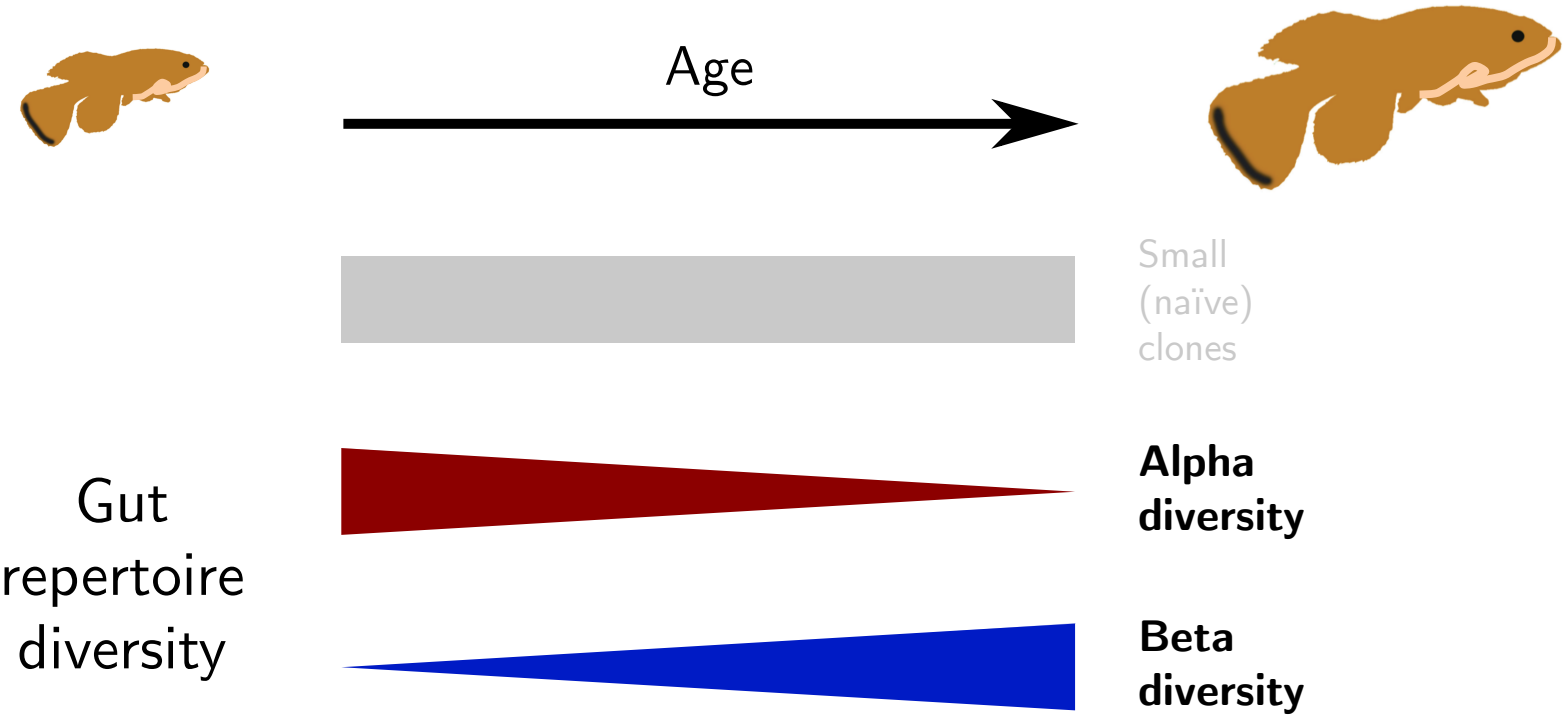

Age
Whole-body repertoire diversity
Small (naïve) clones
Large (expanded) clones
Alpha diversity
Beta diversity

Summary II


Age
Small (naïve) clones
Gut repertoire diversity
Alpha diversity
Beta diversity

Summary II


Age
Small (naïve) clones
Gut repertoire diversity
Alpha diversity
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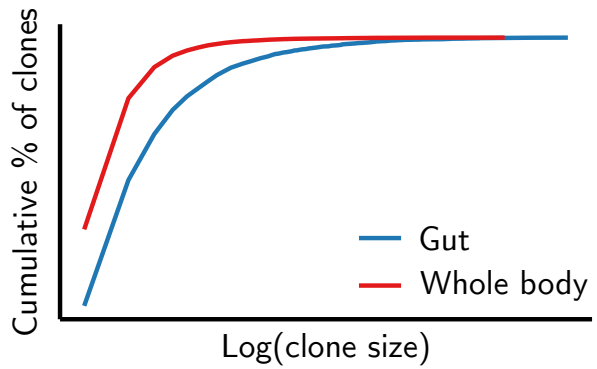
Cumulative % of clones
Log(clone size)
Gut
Whole body

Acknowledgements

Dario Valenzano
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Davina Patel
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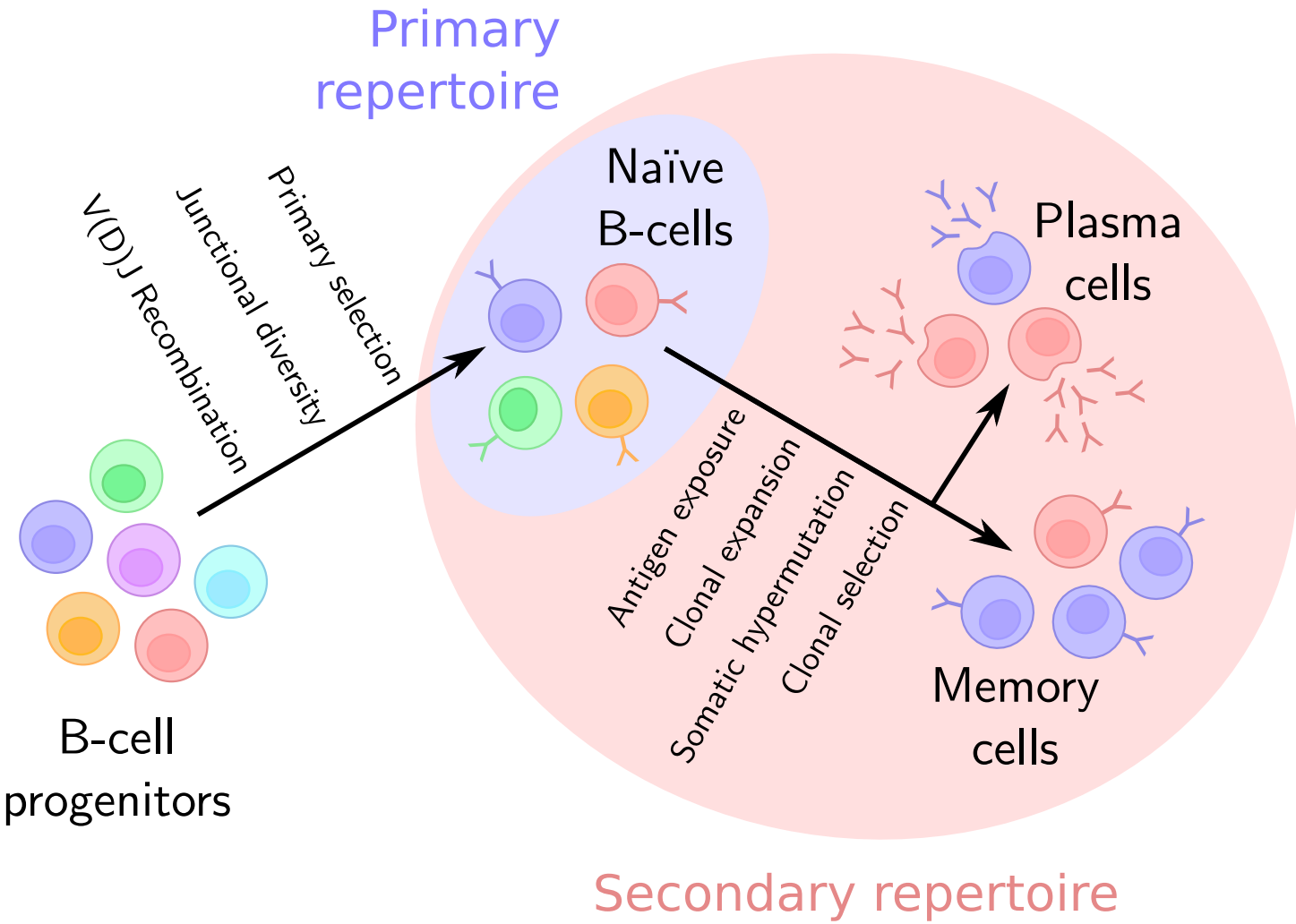
Bérénice Benayoun
John Beausang

Kathrin Reichwald
Jason Vander Heiden
Quentin Marcou

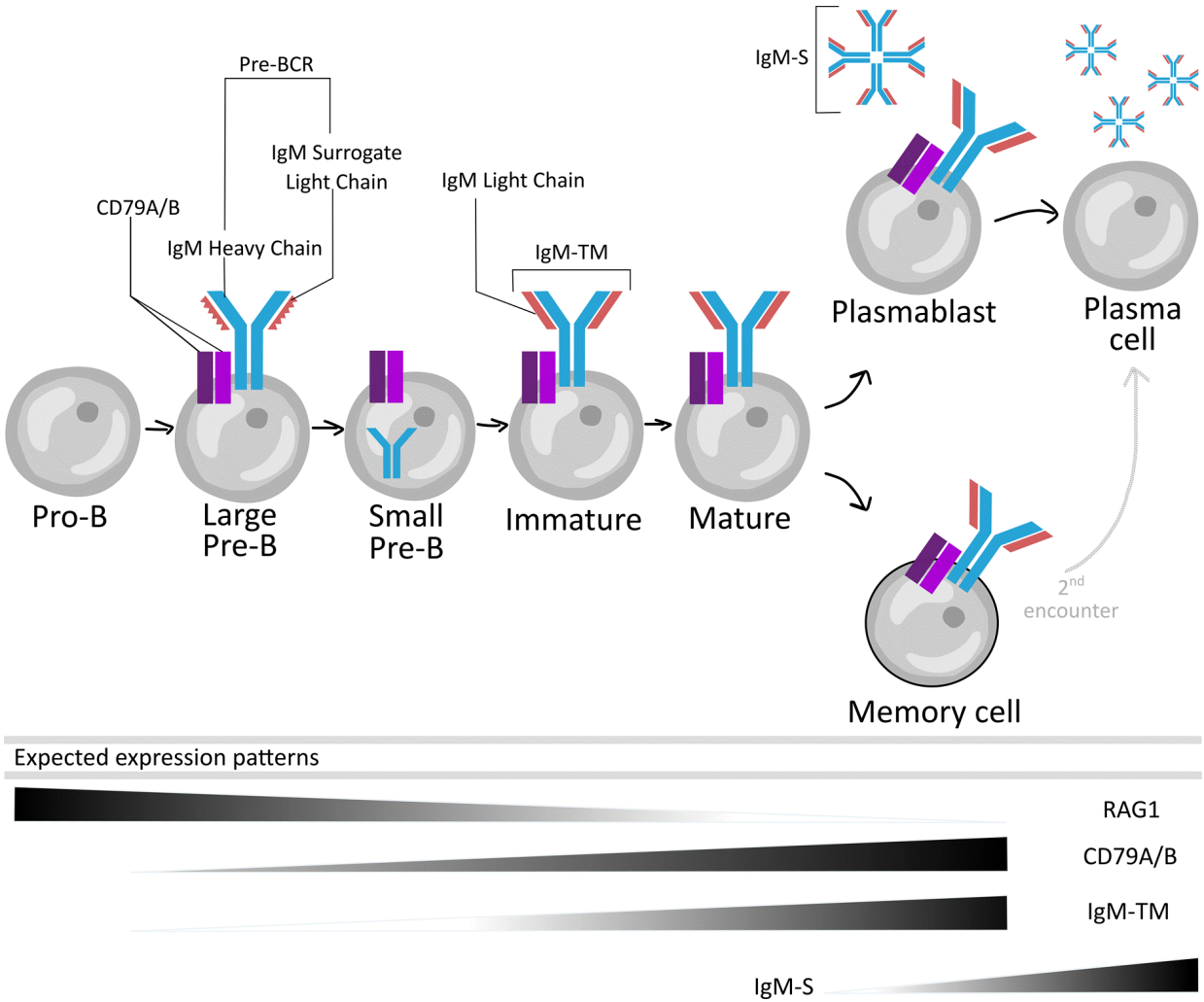
Manolis Pasparakis
Andreas Beyer
Michael Lässig

Thank you!

Primary and secondary antibody diversity

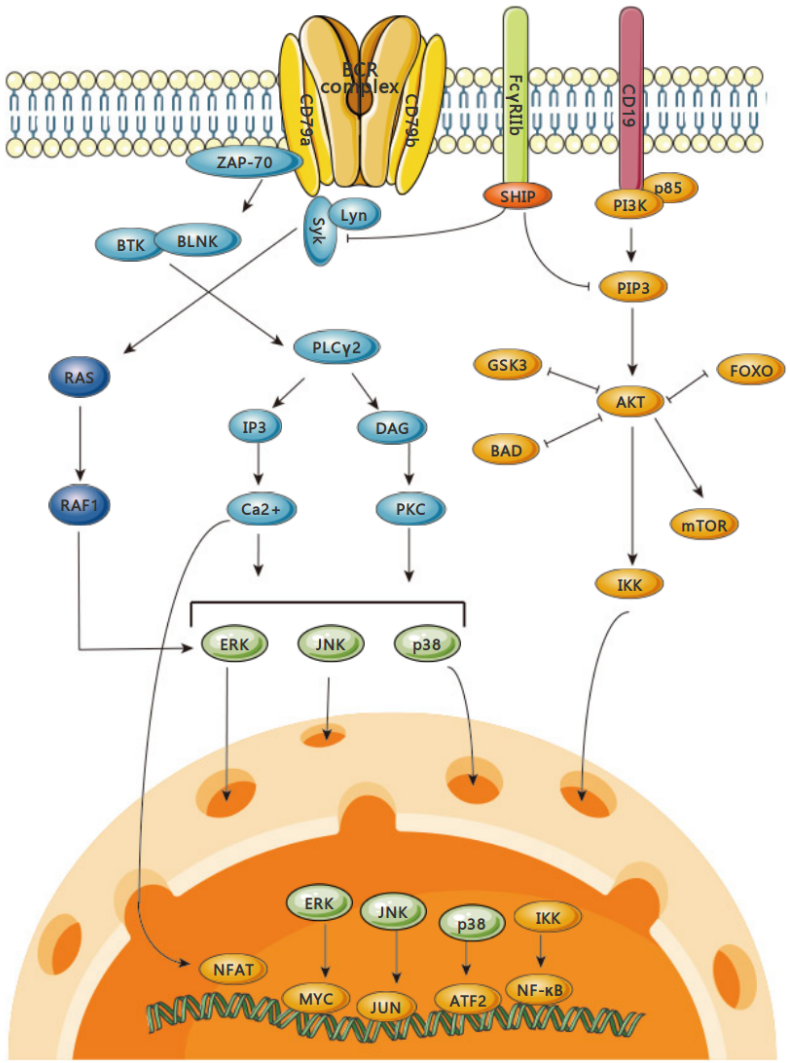


B-cell development



Davina Patel

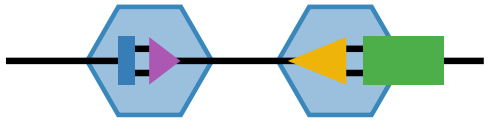
BCR signalling pathway



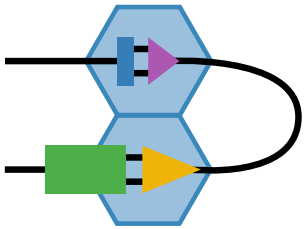
Mechanism of VDJ recombination

(i) Native state

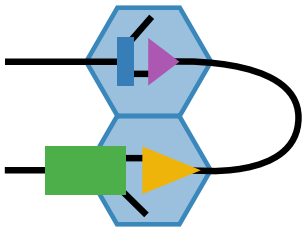
(ii) Recombinase binding



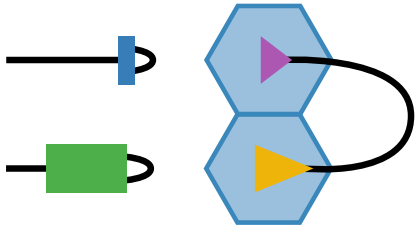
(iii) Pairing



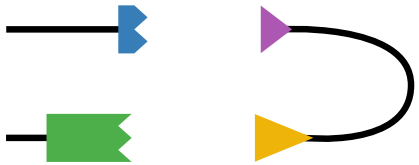
(iv) Single-strand breaks



(v) DSBs & hairpin formation

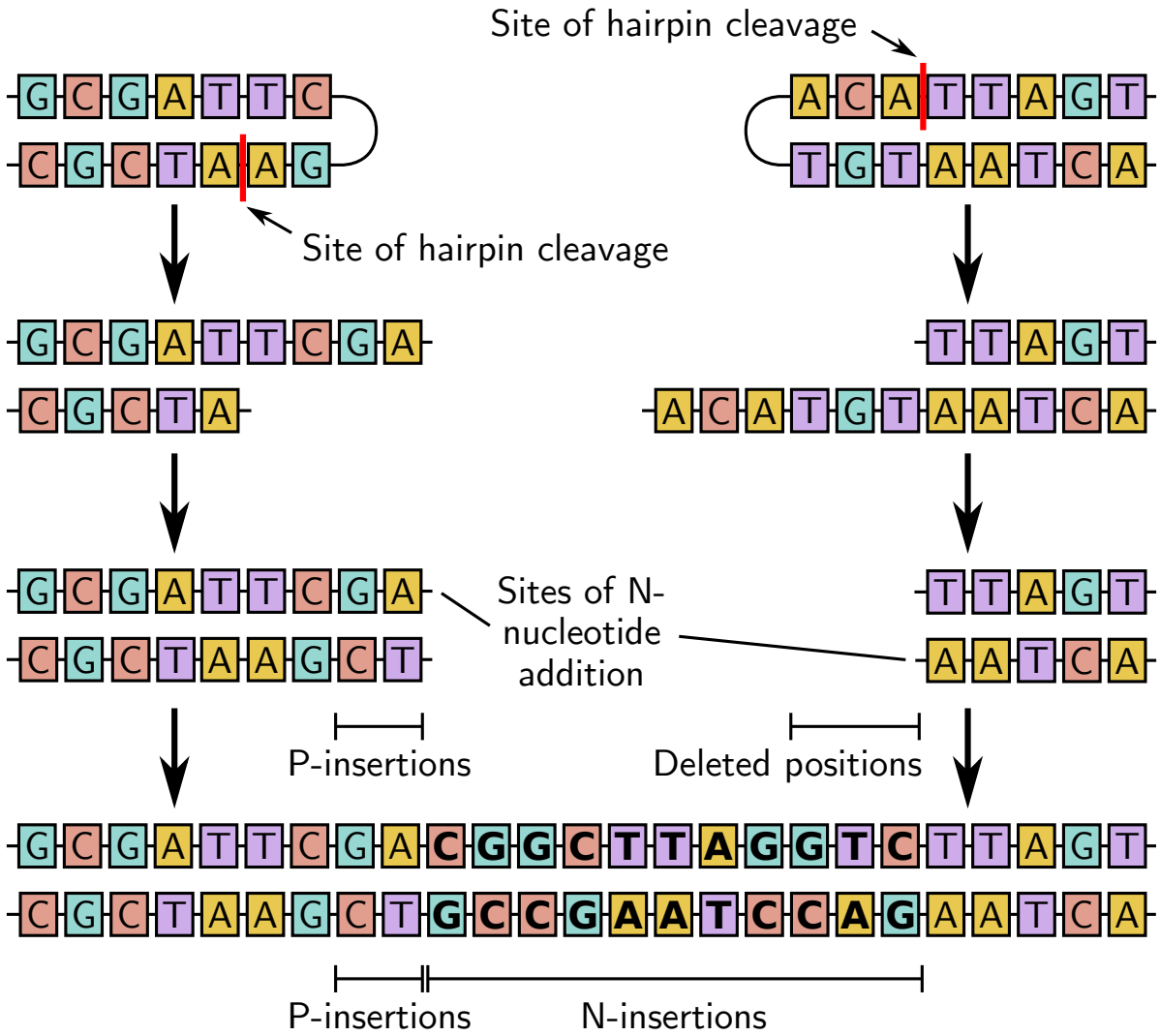


(vi) Hairpin opening & processing



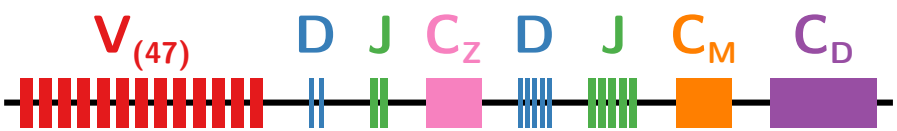
(vii) Resolution

Mechanisms of junctional diversity

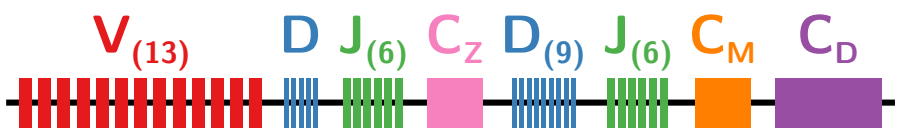


Other teleost loci: simple

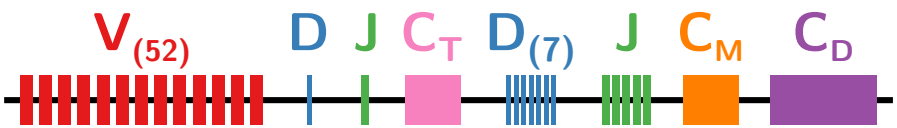
Zebrafish (175 kb)



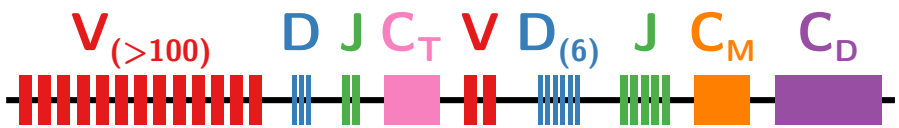
Grass carp (137 kb)



Fugu (130 kb)

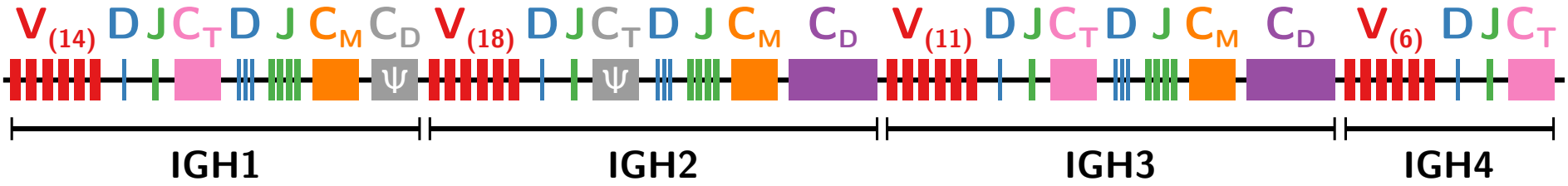


Rainbow trout (125 kb)

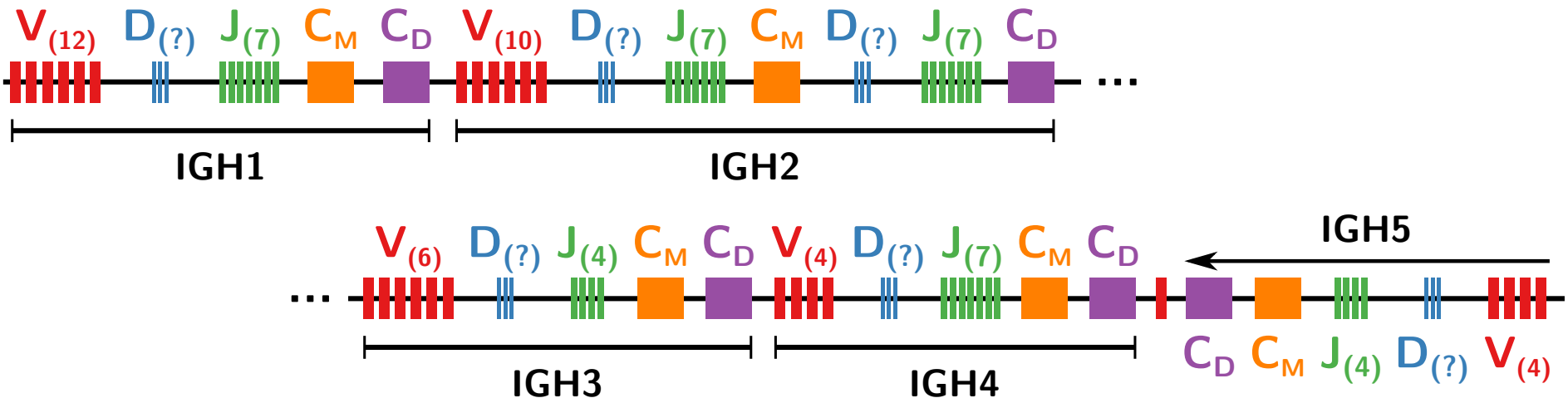


Other teleost loci: complex

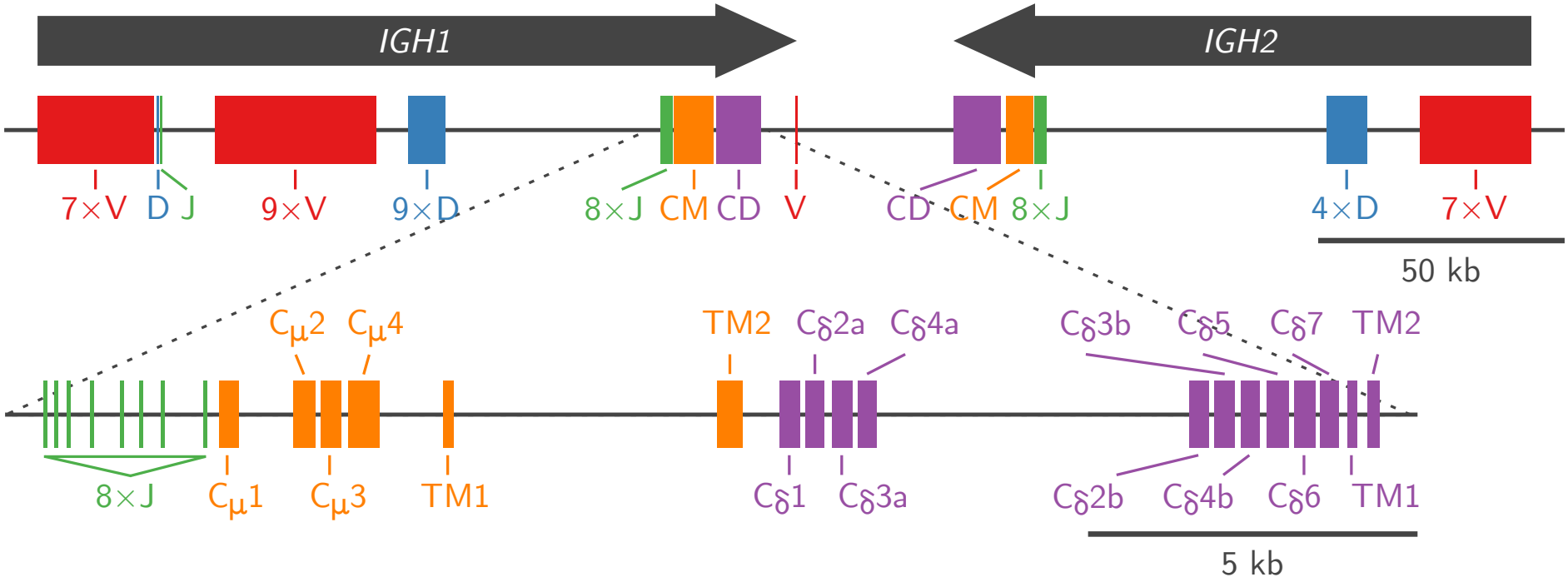
Three-spined stickleback (200 kb)



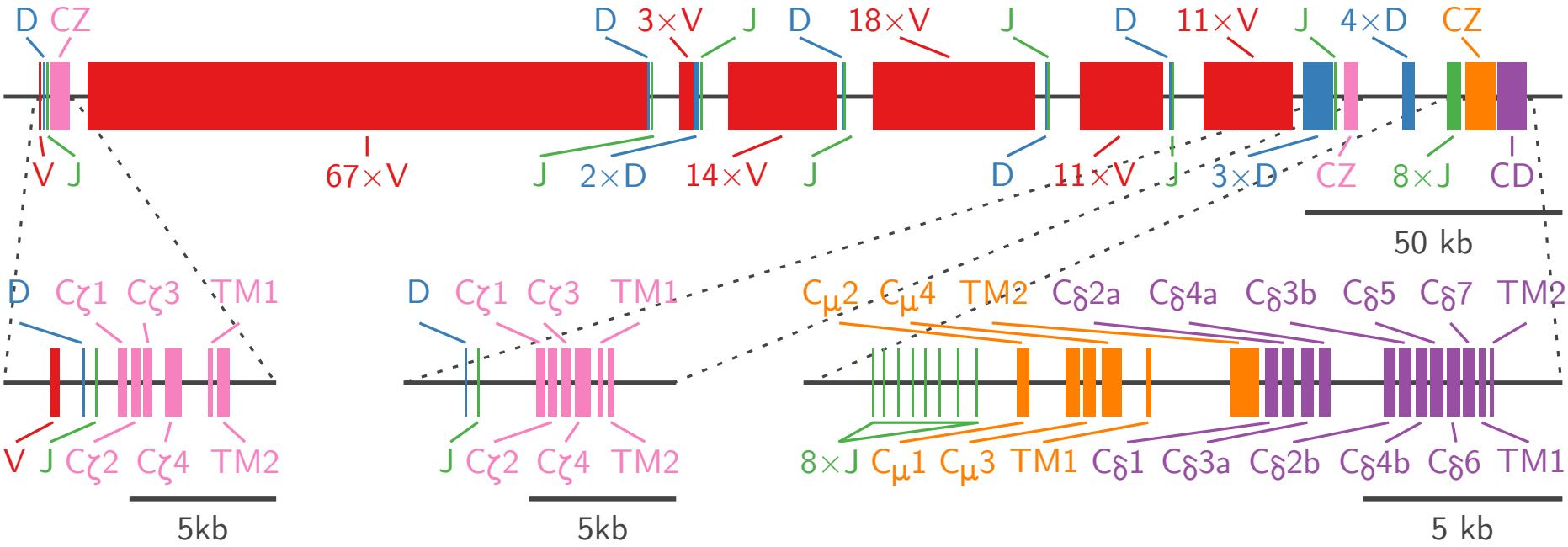
Medaka (450 kb)

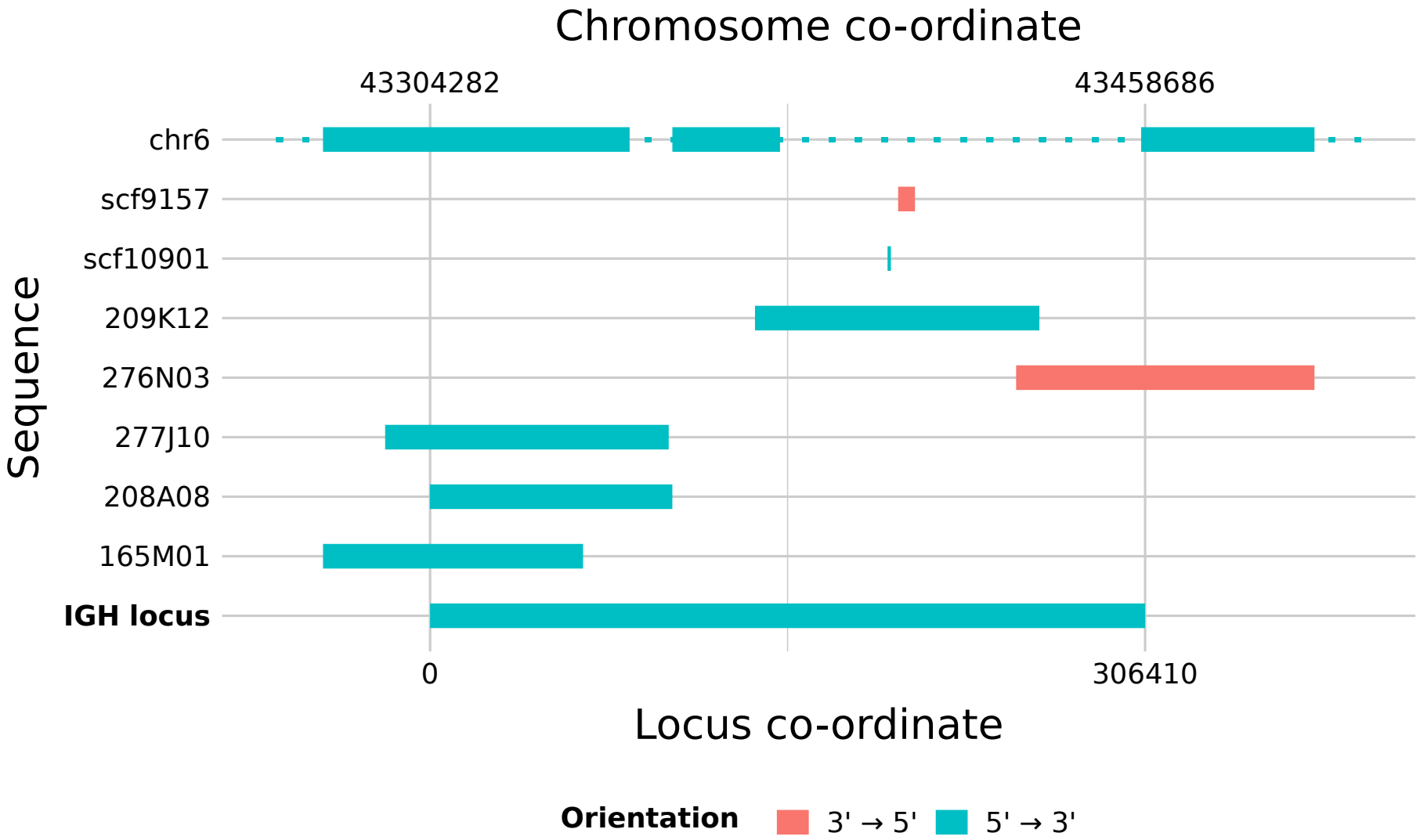


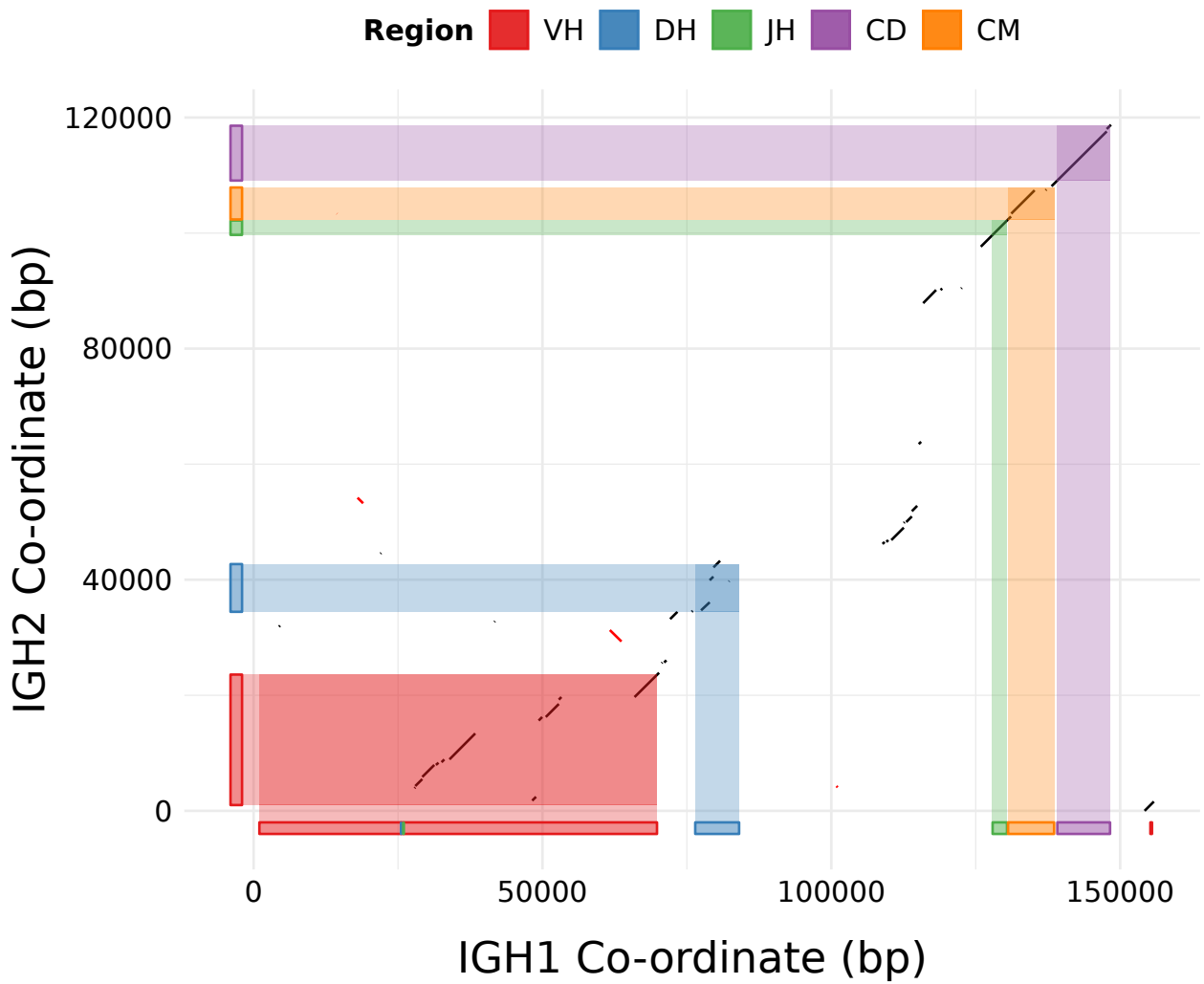
Nothobranchius furzeri IGH

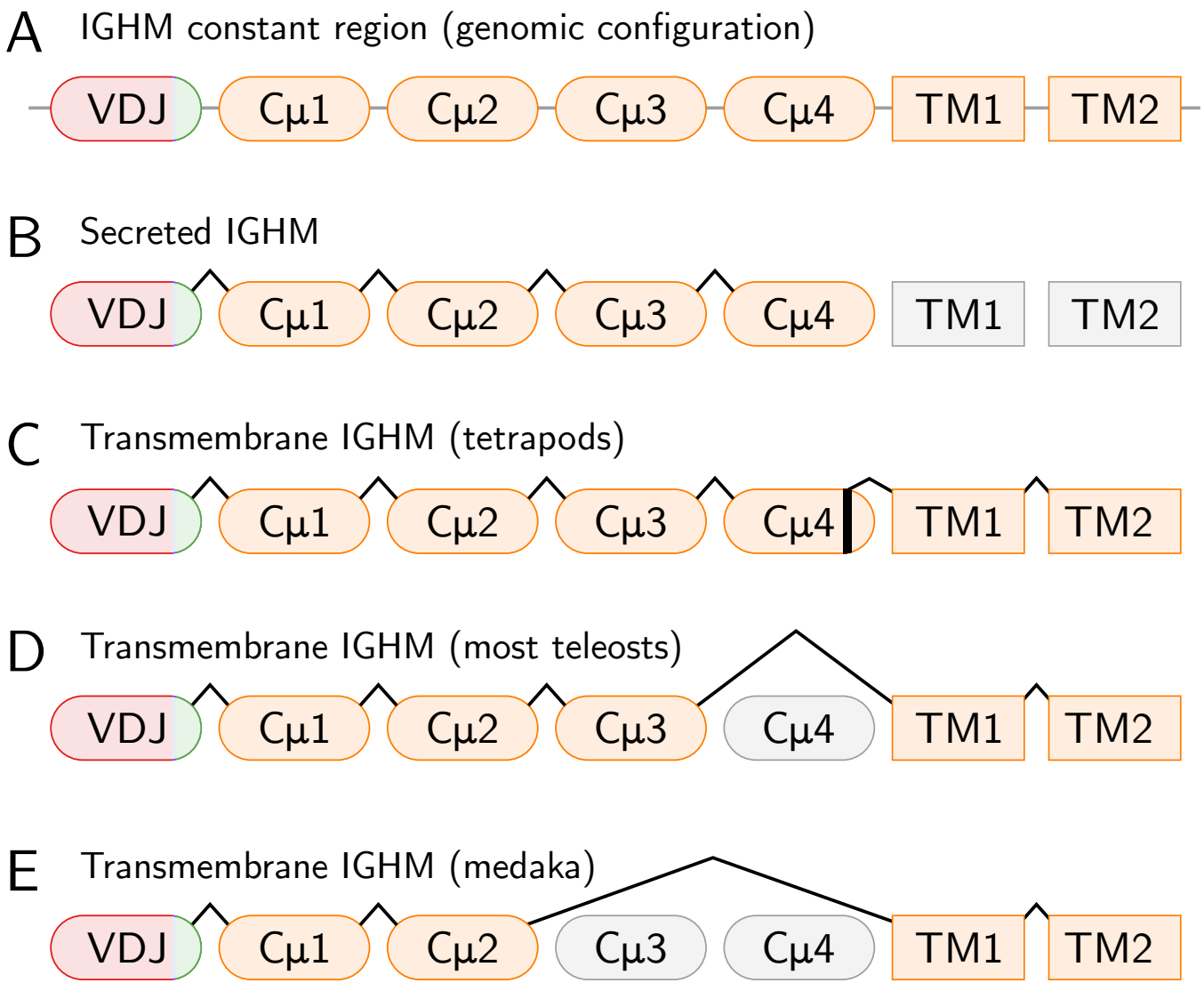


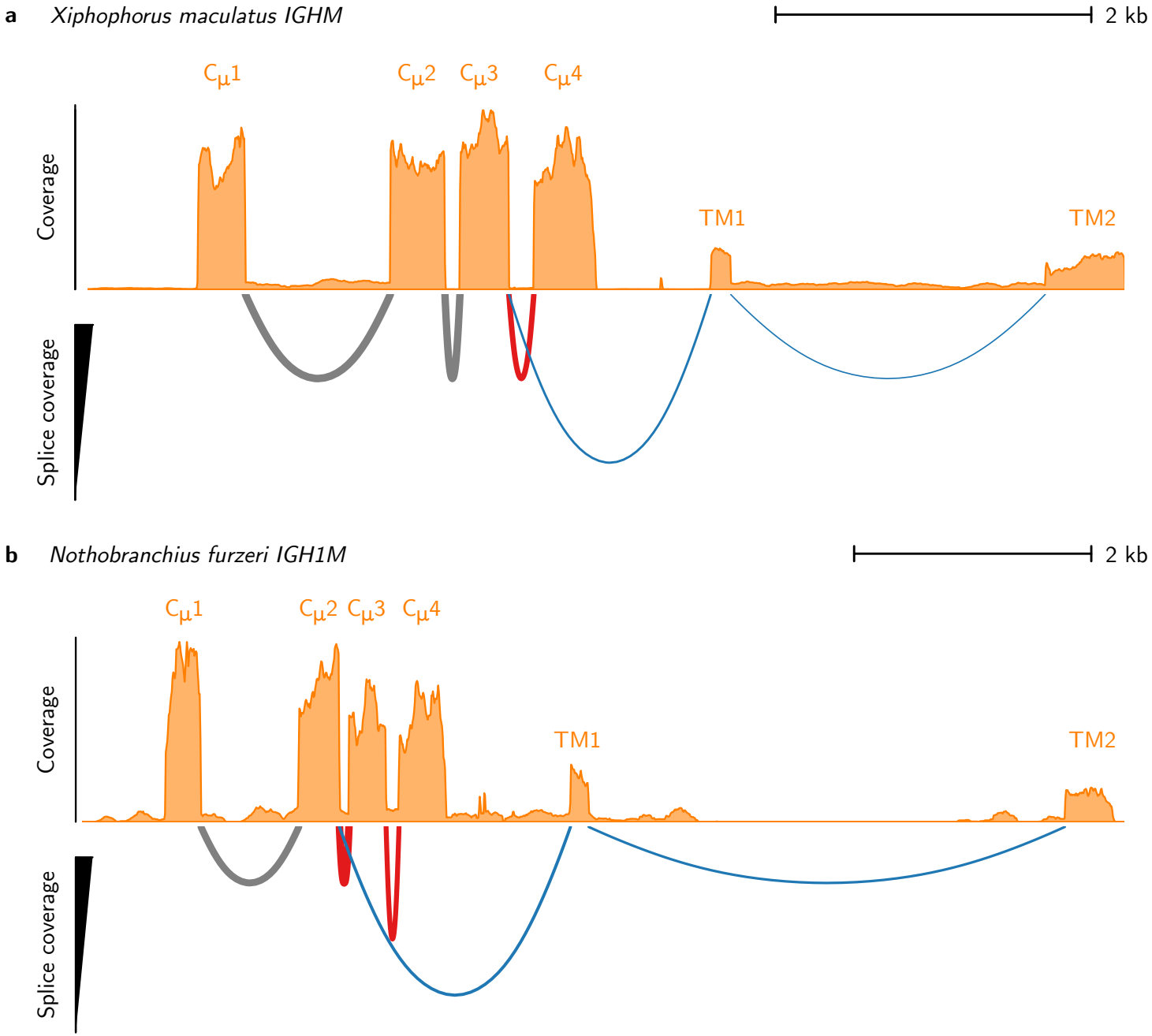
Xiphophorus maculatus IGH

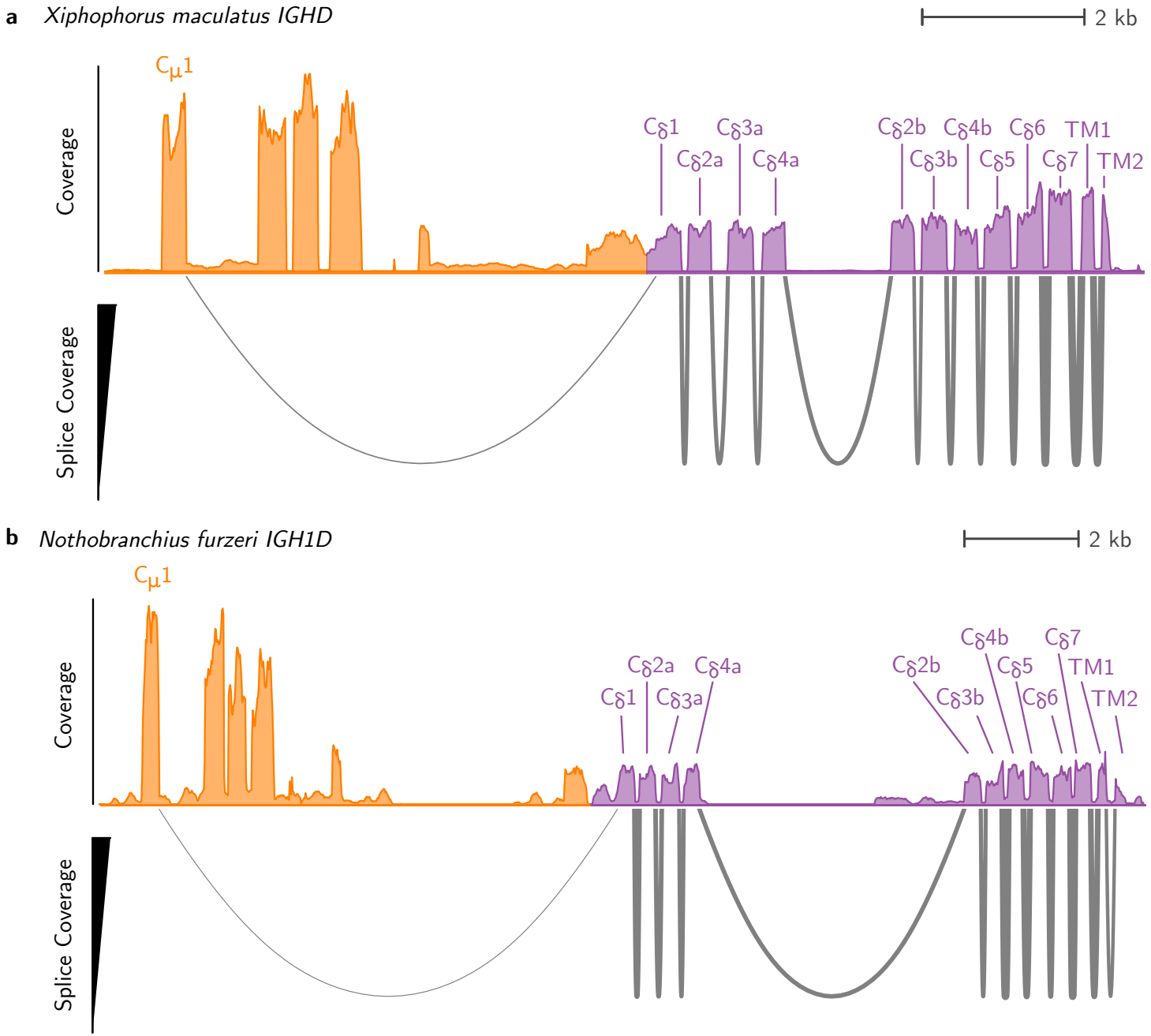


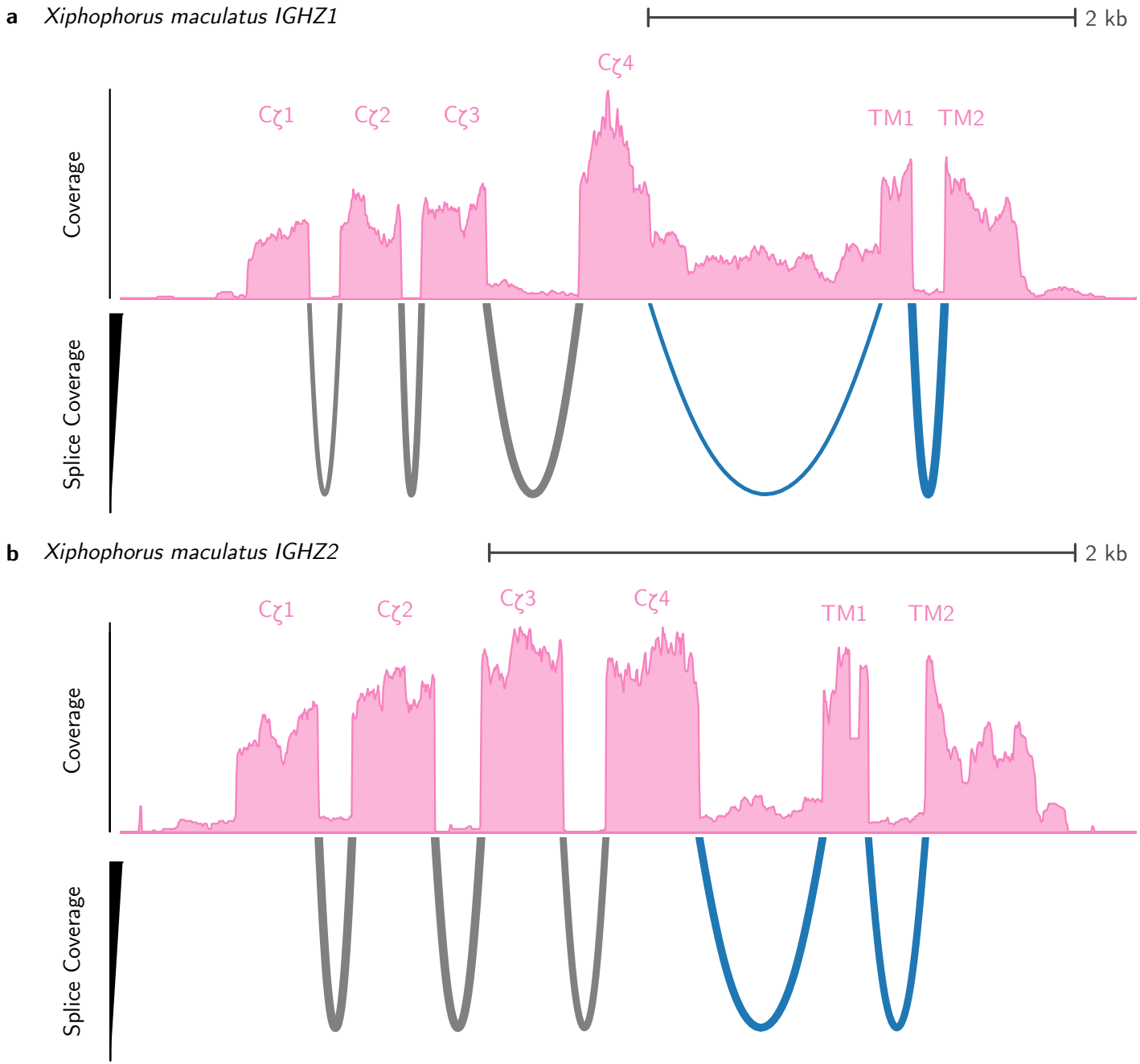

Chromosome co-ordinate
43304282
43458686
chr6
scf9157
scf10901
209K12
276N03
277J10
208A08
165M01
IGH locus
Sequence
0
306410
Locus co-ordinate
Orientation
3' → 5'
5' → 3'


Region
VH
DH
JH
CD
CM
120000
80000
40000
0
IGH2 Co-ordinate (bp)
0
50000
100000
150000
IGH1 Co-ordinate (bp)

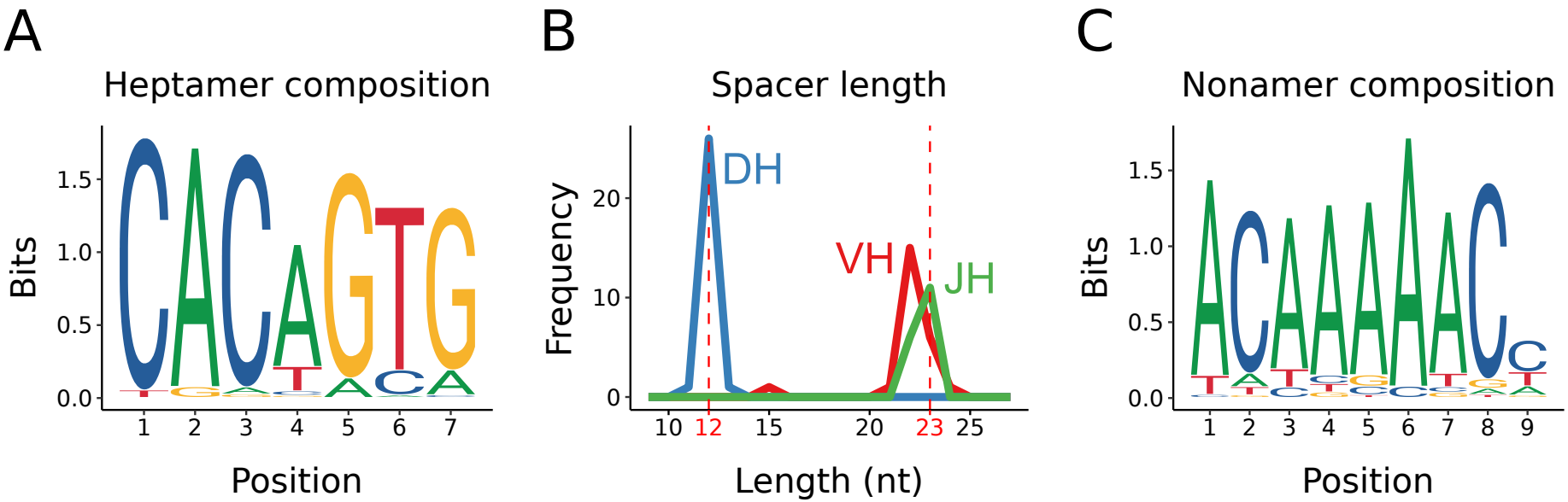

A
IGHM constant region (genomic configuration)
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TM1
TM2
B
Secreted IGHM
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TM1
TM2
C
Transmembrane IGHM (tetrapods)
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TM1
TM2
D
Transmembrane IGHM (most teleosts)
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TM1
TM2
E
Transmembrane IGHM (medaka)
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TM1
TM2


a
Xiphophorus maculatus IGHM
2 kb
Cμ1
Cμ2
Cμ3
Cμ4
Coverage
TM1
TM2
Splice coverage
b
Nothobranchius furzeri IGH1M
2 kb
Cμ1
Cμ2
Cμ3
Cμ4
Coverage
TM1
TM2
Splice coverage

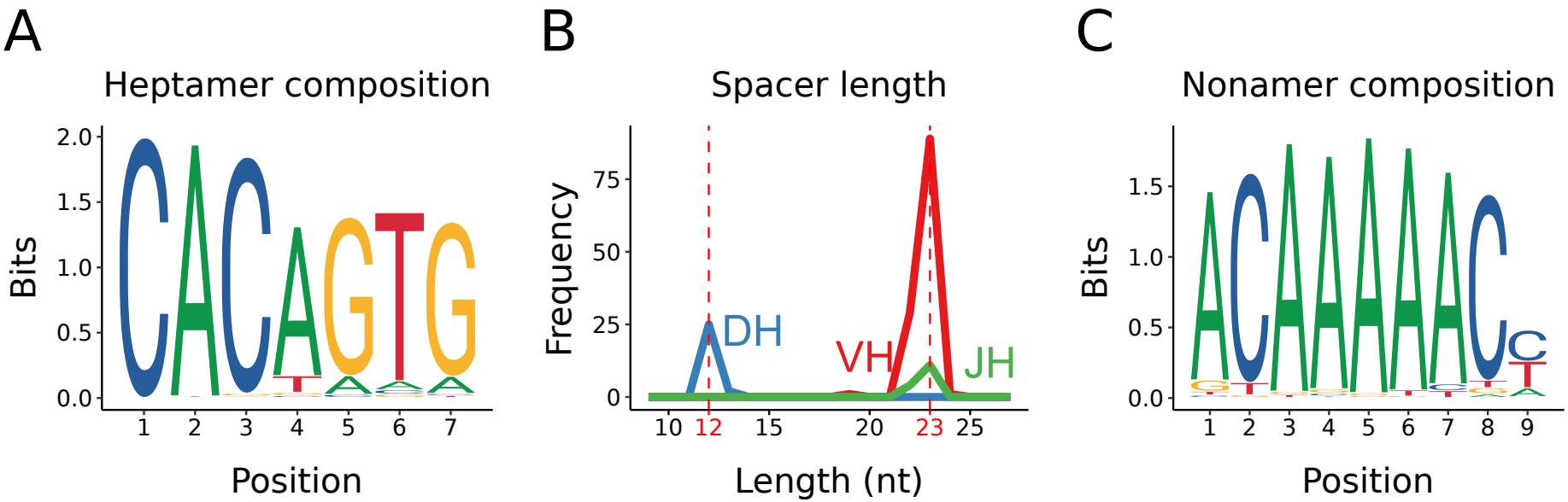

a
Xiphophorus maculatus IGHD
2 kb
Cμ1
Cδ1
Cδ2a
Cδ3a
Cδ4a
Cδ2b
Cδ3b
Cδ4b
Cδ5
Cδ6
Cδ7
TM1
TM2
Coverage
Splice Coverage
b
Nothobranchius furzeri IGH1D
2 kb
Cμ1
Cδ1
Cδ2a
Cδ3a
Cδ4a
Cδ2b
Cδ3b
Cδ4b
Cδ5
Cδ6
Cδ7
TM1
TM2
Coverage
Splice Coverage

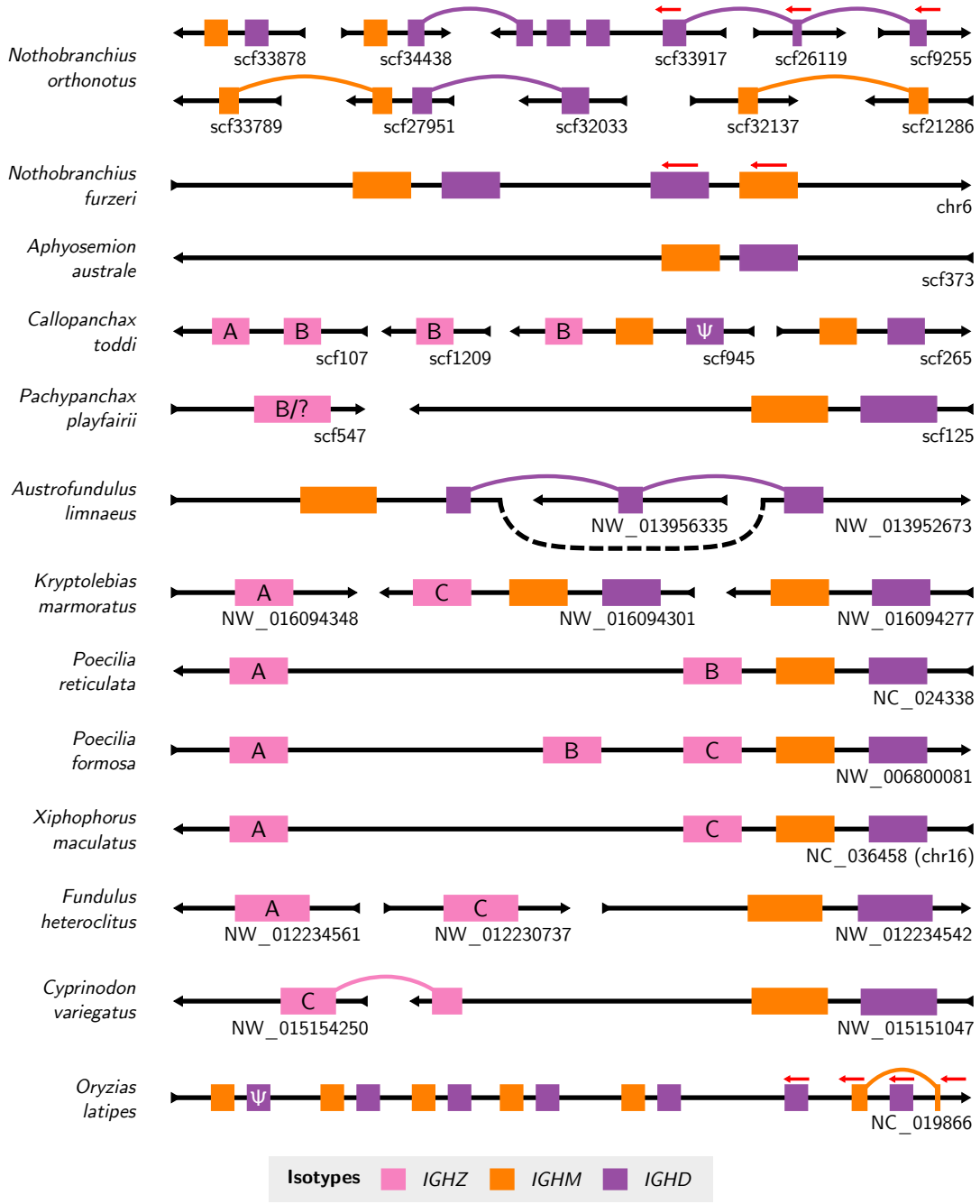

a
Xiphophorus maculatus IGHZ1
2 kb
Cζ4
Cζ1
Cζ2
Cζ3
TM1
TM2
Coverage
Splice Coverage
b
Xiphophorus maculatus IGHZ2
2 kb
Cζ1
Cζ2
Cζ3
Cζ4
TM1
TM2
Coverage
Splice Coverage

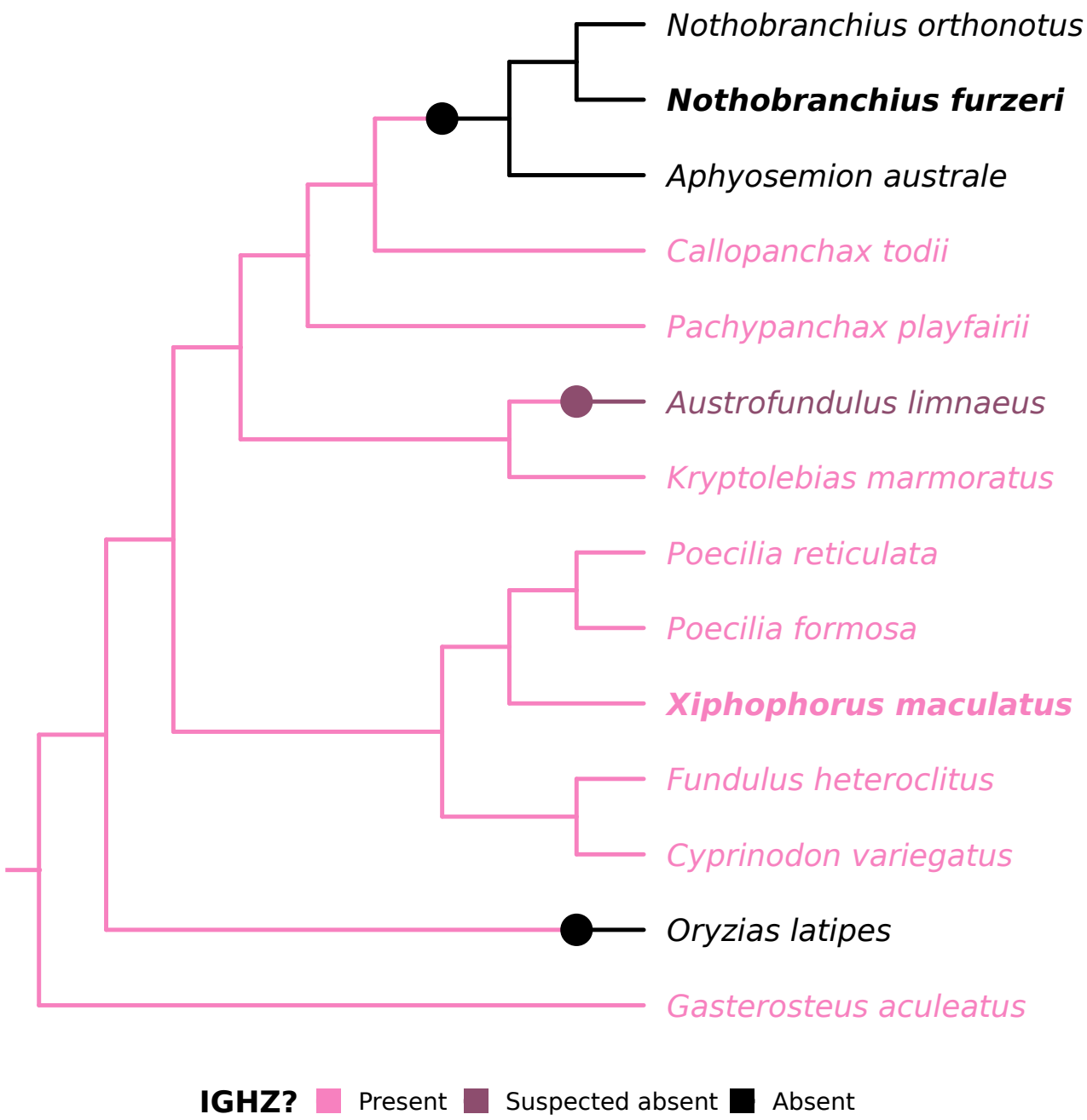
N. furzeri RSS composition

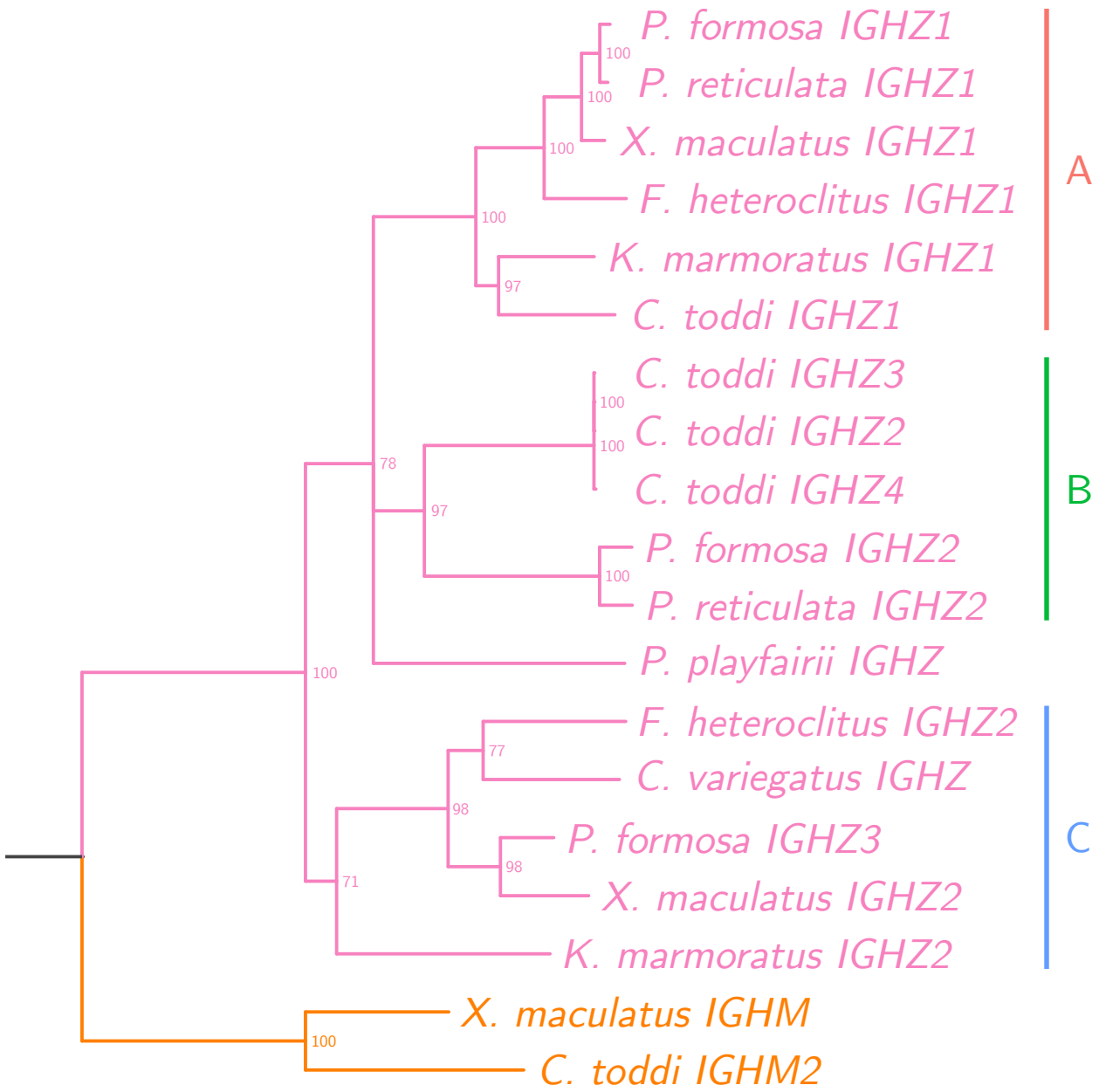


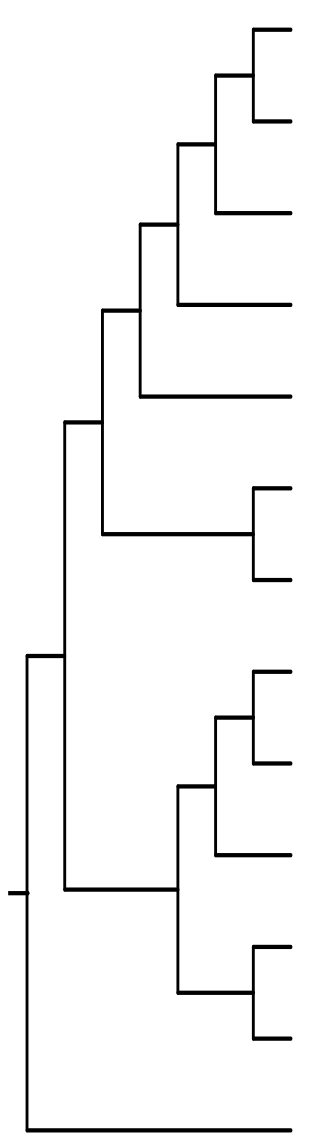
X. maculatus RSS composition



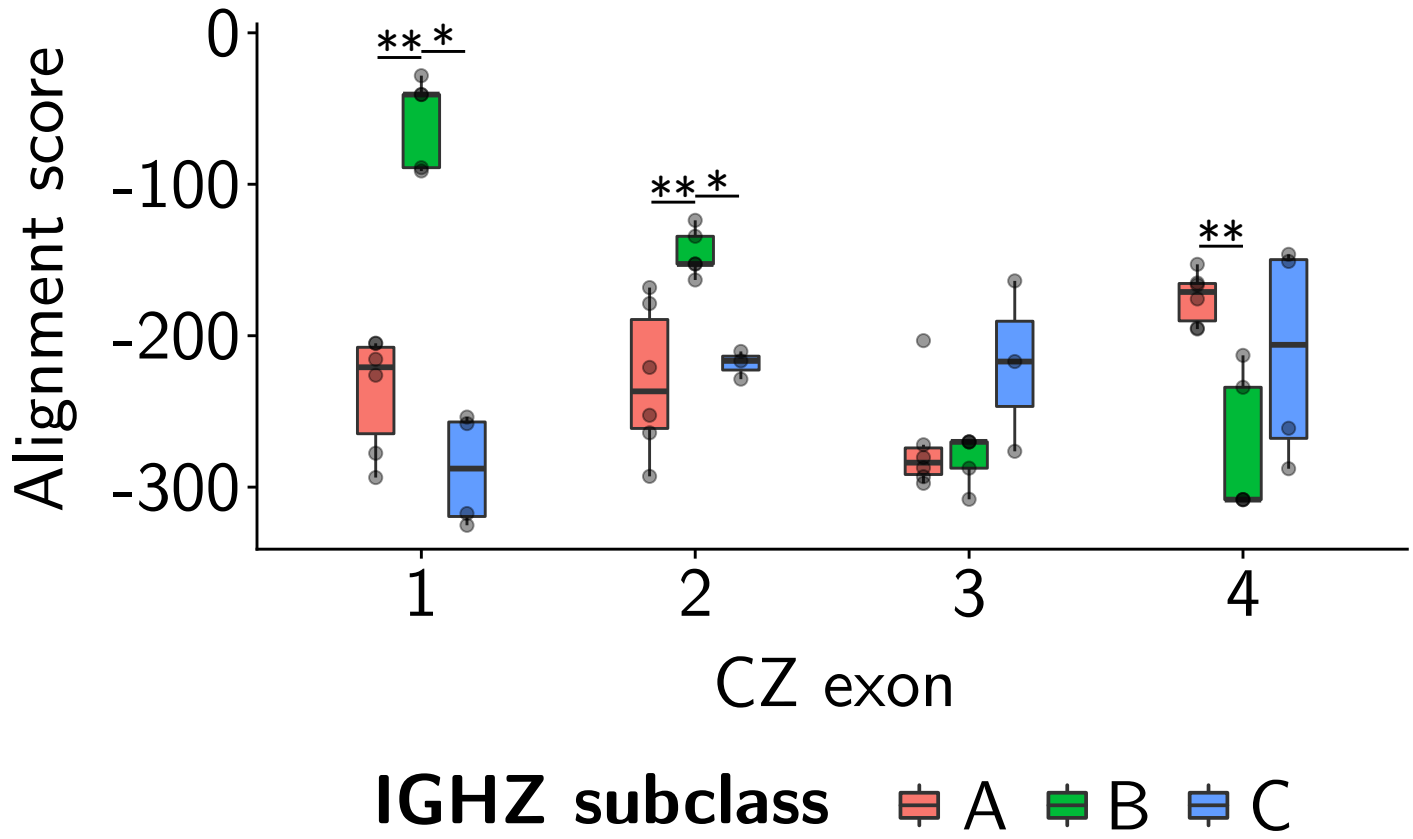

Nothobranchius orthonotus
scf33878
scf34438
scf33917
scf26119
scf9255
scf33789
scf27951
scf32033
scf32137
scf21286
Nothobranchius furzeri
chr6
Aphyosemion australe
scf373
Callopanchax toddi
A
B
B
B
Ψ
scf107
scf1209
scf945
scf265
Pachypanchax playfairii
B/?
scf547
scf125
Austrofundulus limnaeus
NW_013956335
NW_013952673
Kryptolebias marmoratus
A
C
NW_016094348
NW_016094301
NW_016094277
Poecilia reticulata
A
B
NC_024338
Poecilia formosa
A
B
C
NW_006800081
Xiphophorus maculatus
A
C
NC_036458 (chr16)
Fundulus heteroclitus
A
C
NW_012234561
NW_012230737
NW_012234542
Cyprinodon variegatus
C
NW_015154250
NW_015151047
Oryzias latipes
Ψ
NC_019866
Isotypes
IGHZ
IGHM
IGHD

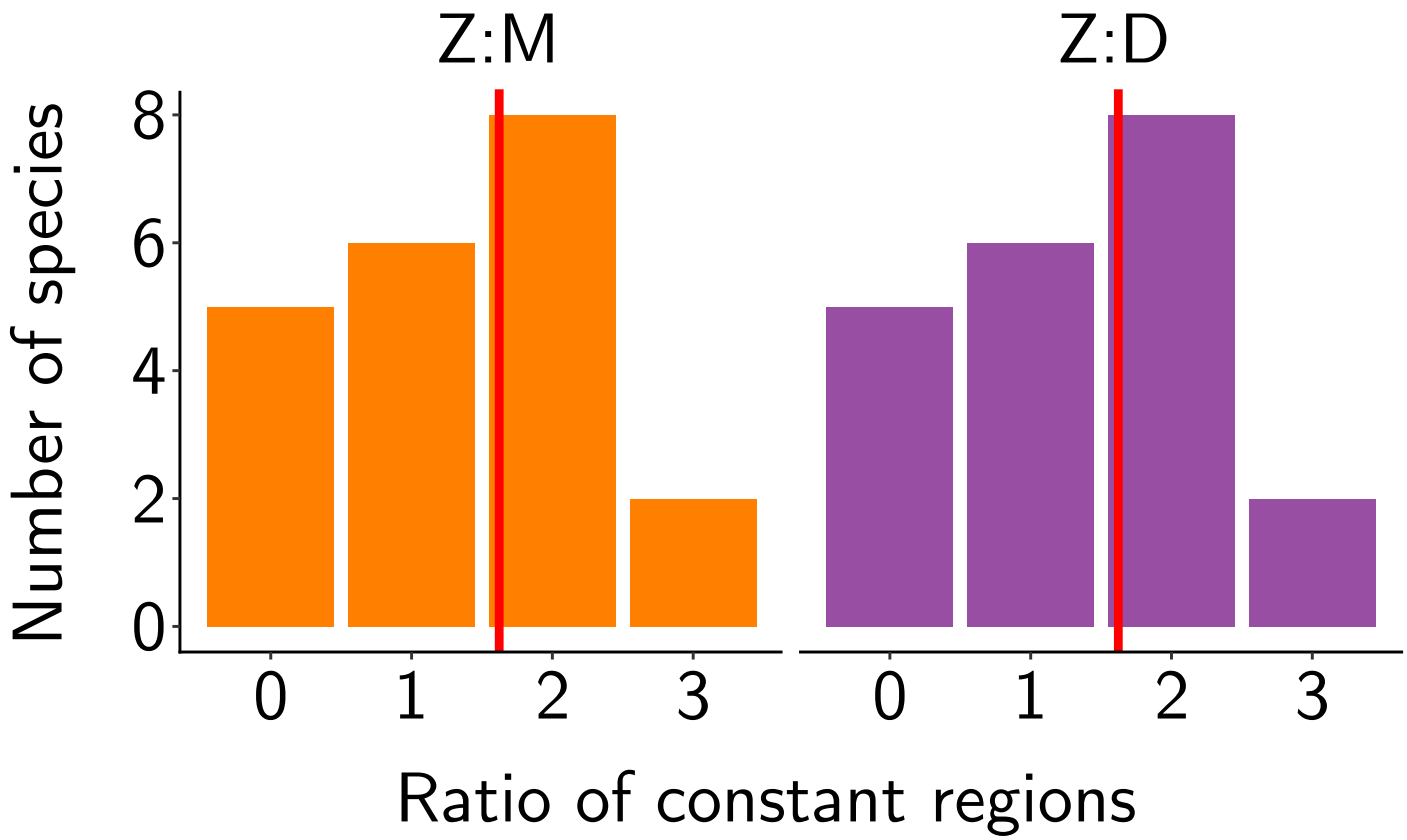

Nothobranchius orthonotus
Nothobranchius furzeri
Aphyosemion australe
Callopanchax todii
Pachypanchax playfairii
Austrofundulus limnaeus
Kryptolebias marmoratus
Poecilia reticulata
Poecilia formosa
Xiphophorus maculatus
Fundulus heteroclitus
Cyprinodon variegatus
Oryzias latipes
Gasterosteus aculeatus
IGHZ?
Present
Suspected absent
Absent


P. formosa IGHZ1
P. reticulata IGHZ1
X. maculatus IGHZ1
F. heteroclitus IGHZ1
K. marmoratus IGHZ1
C. toddi IGHZ1
C. toddi IGHZ3
C. toddi IGHZ2
C. toddi IGHZ4
P. formosa IGHZ2
P. reticulata IGHZ2
P. playfairii IGHZ
F. heteroclitus IGHZ2
C. variegatus IGHZ
P. formosa IGHZ3
X. maculatus IGHZ2
K. marmoratus IGHZ2
X. maculatus IGHM
C. toddi IGHM2
A
B
C
100
100
100
100
97
78
97
100
100
100
77
98
98
71
100

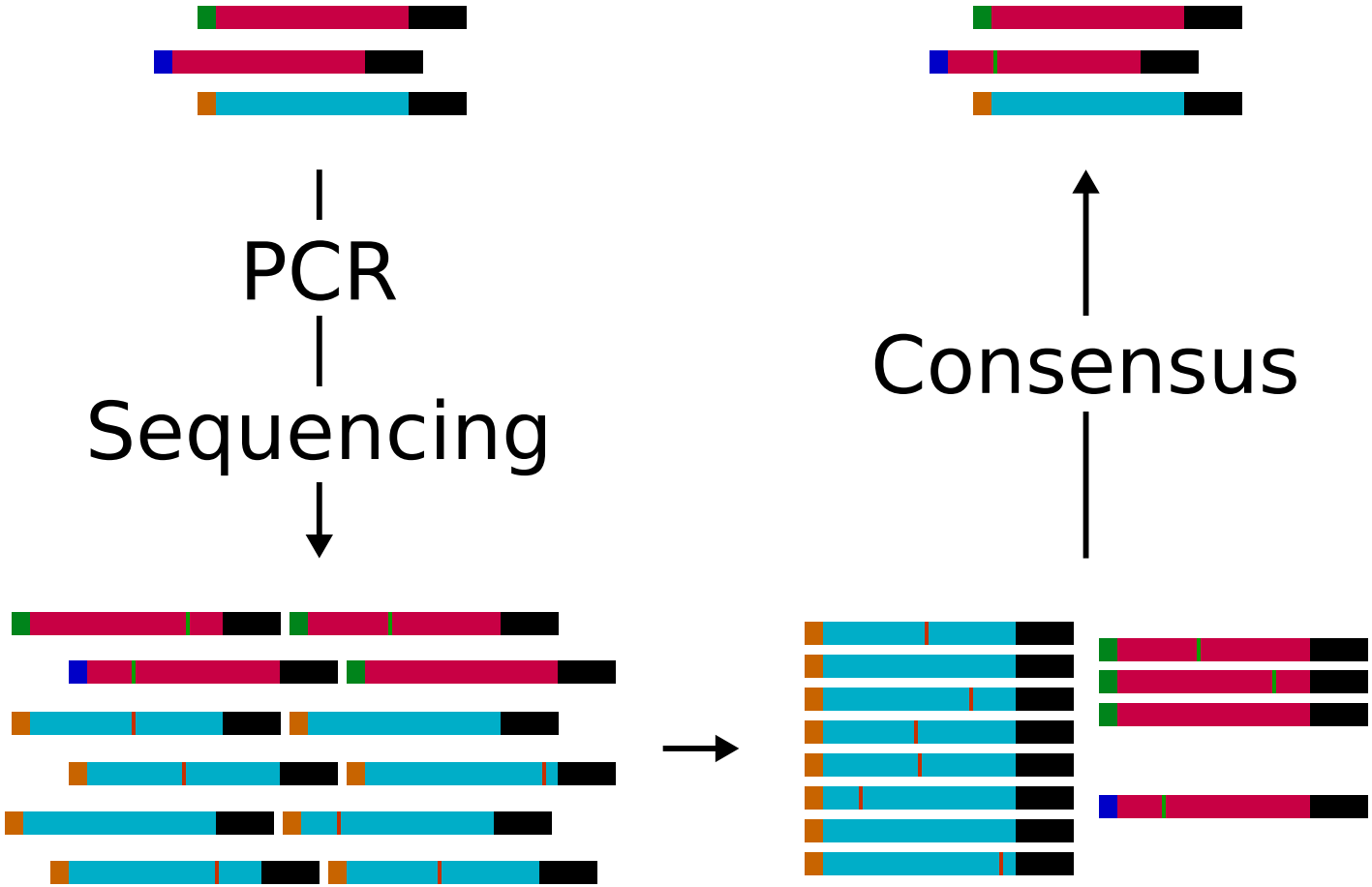


Tree	Species	# *IGHZ-A*	# *IGHZ-B*	# *IGHZ-C*
	N. orthonotus	0	0	0
	N. furzeri	0	0	0
	A. australe	0	0	0
	C. toddi	1	0	3
	P. playfairii	Unknown	1	Unknown
	A. limnaeus	0	0	0
	K. marmoratus	1	0	1
	P. reticulata	1	1	0
	P. formosa	1	1	1
	X. maculatus	1	0	1
	F. heteroclitus	1	0	1
	C. variegatus	0	0	1
	O. latipes	0	0	0

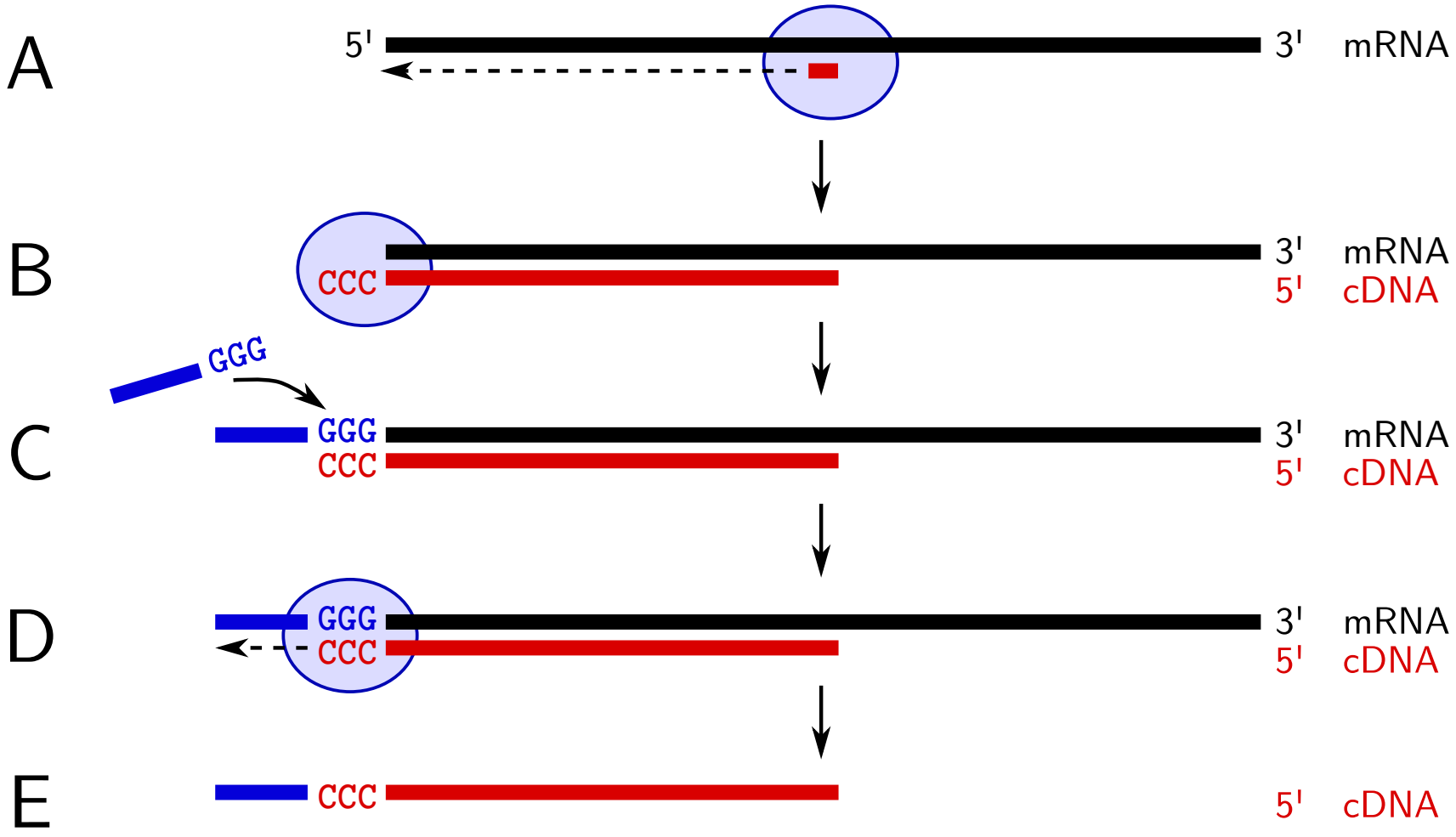

Alignment score
0
-100
-200
-300
**
*
**
*
**
1
2
3
4
CZ exon
IGHZ subclass
A
B
C


Z:M
Z:D
Number of species
0
2
4
6
8
0
1
2
3
0
1
2
3
Ratio of constant regions

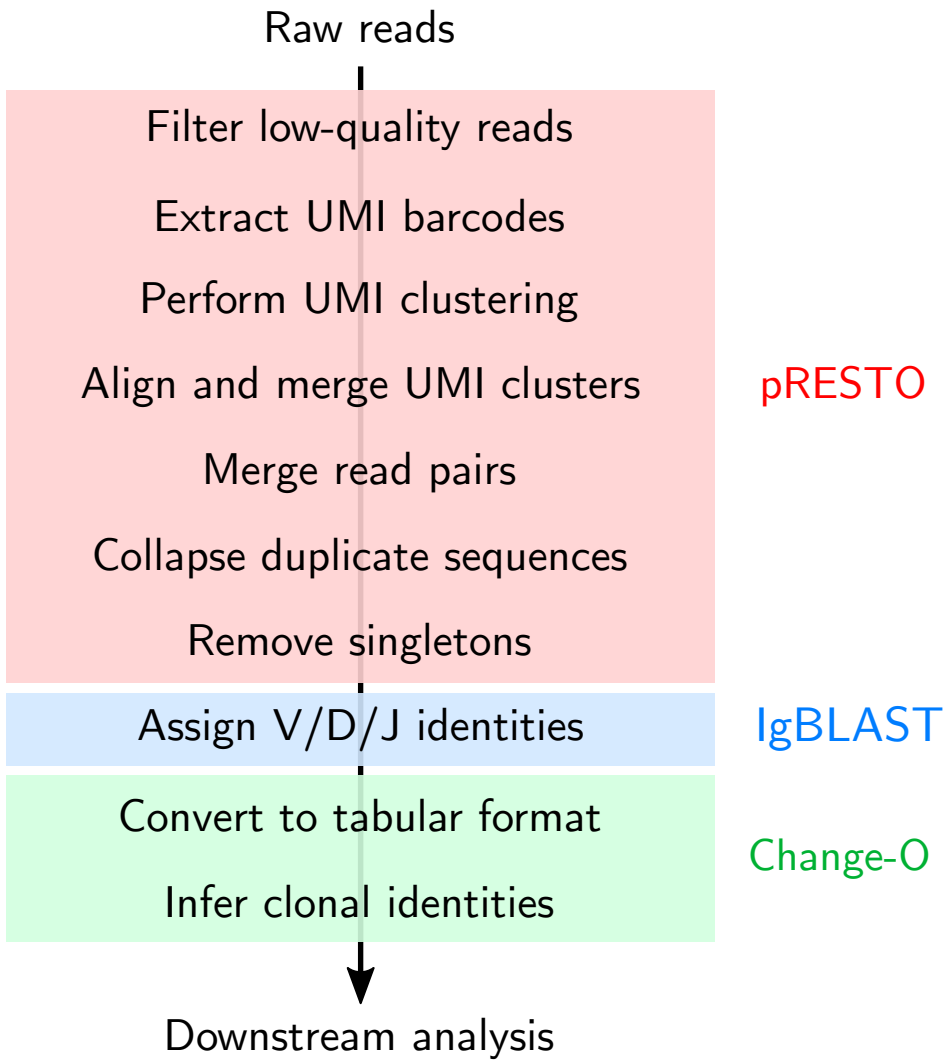
Error correction with UMIs



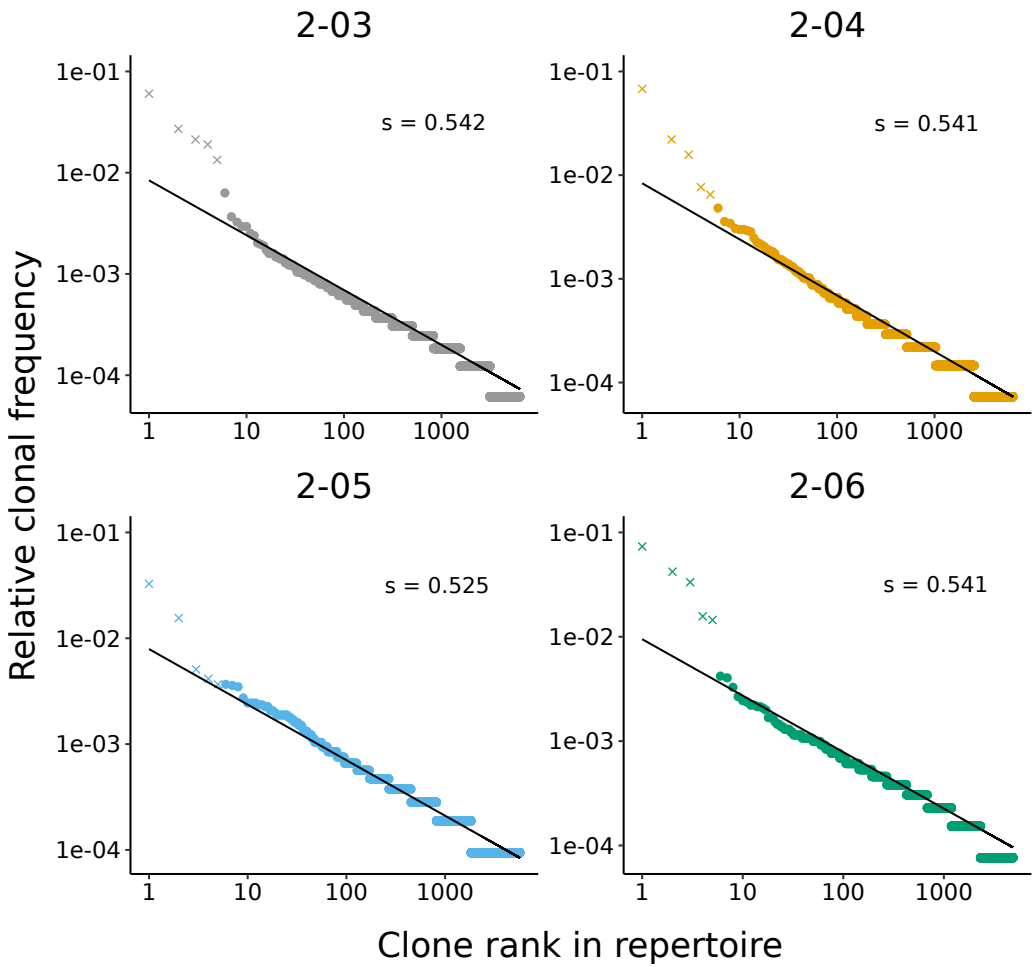
Template switching



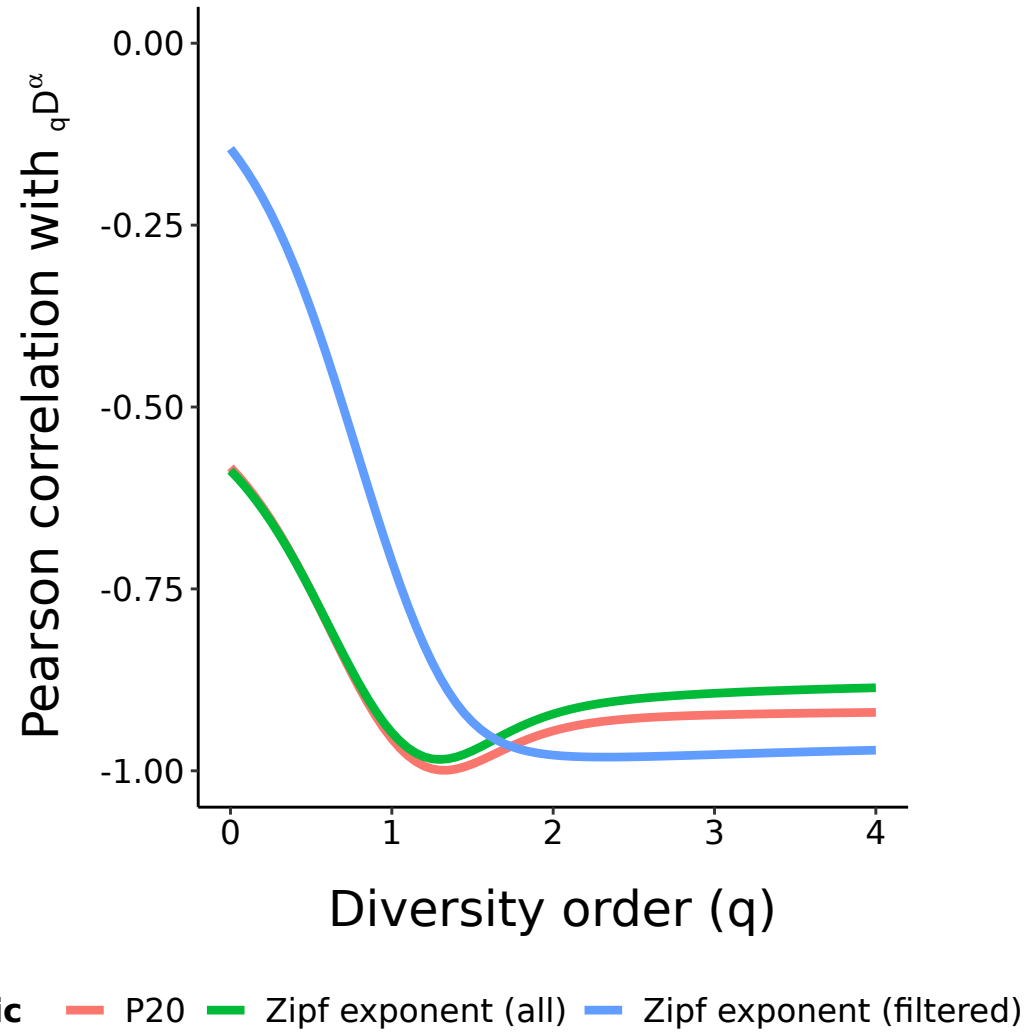
Bioinformatics pipeline



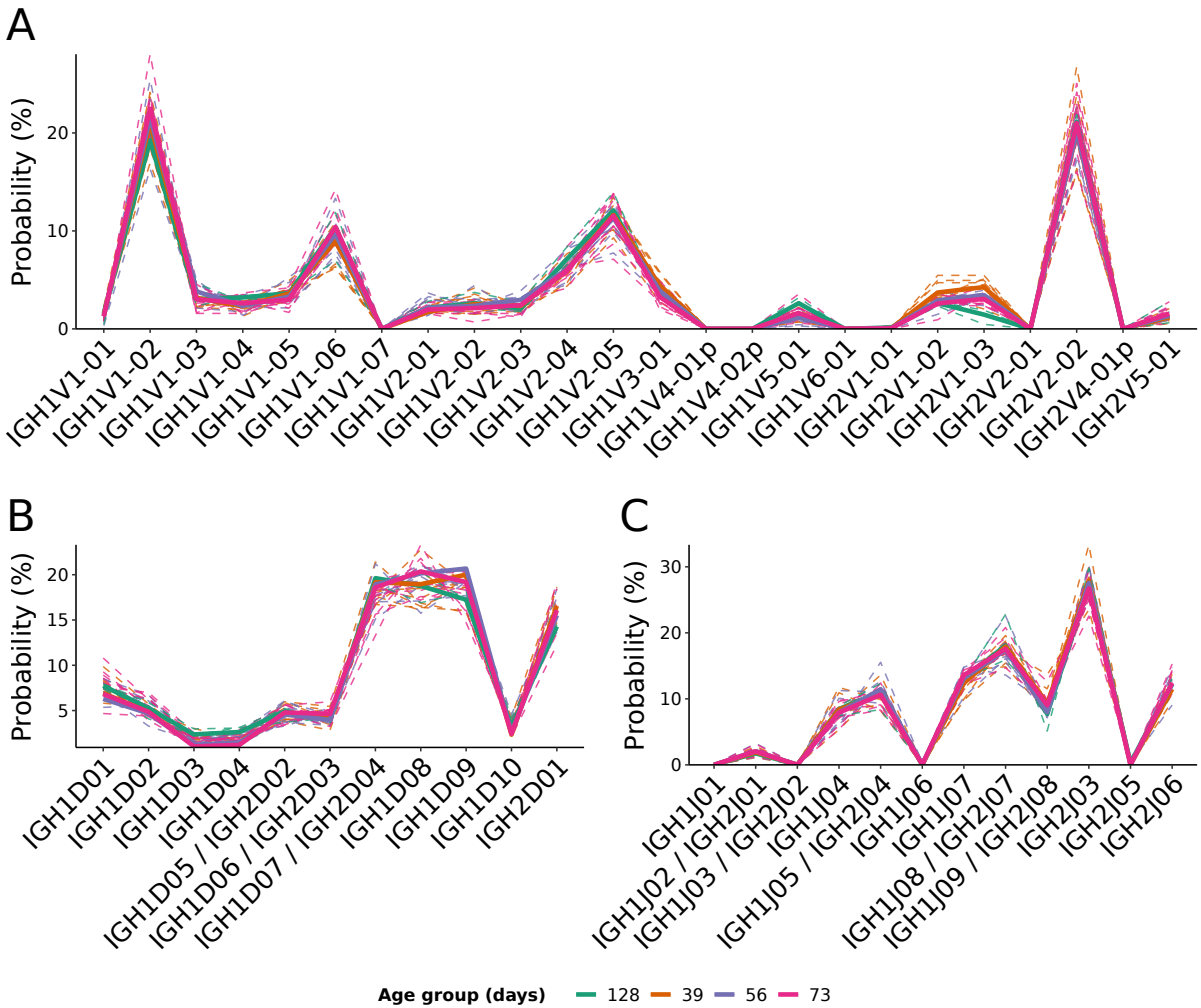
Best-fit zipf distributions of pilot clonal repertoires



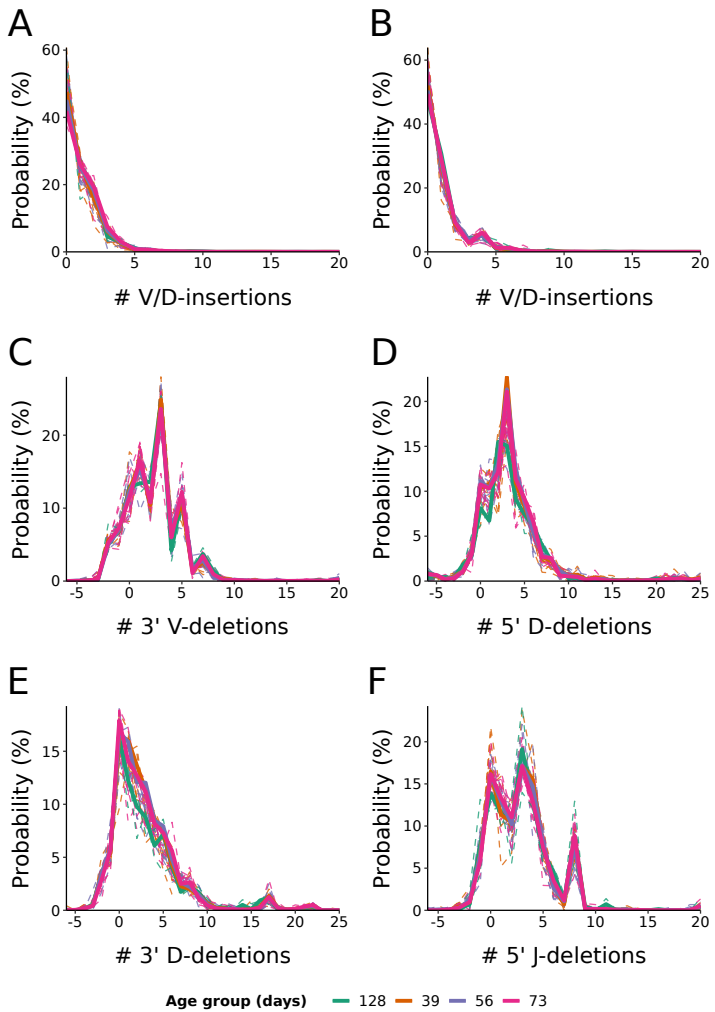
Correlation between Hill α-spectra and other diversity metrics



V/D/J usage probabilities in the killifish generative repertoire

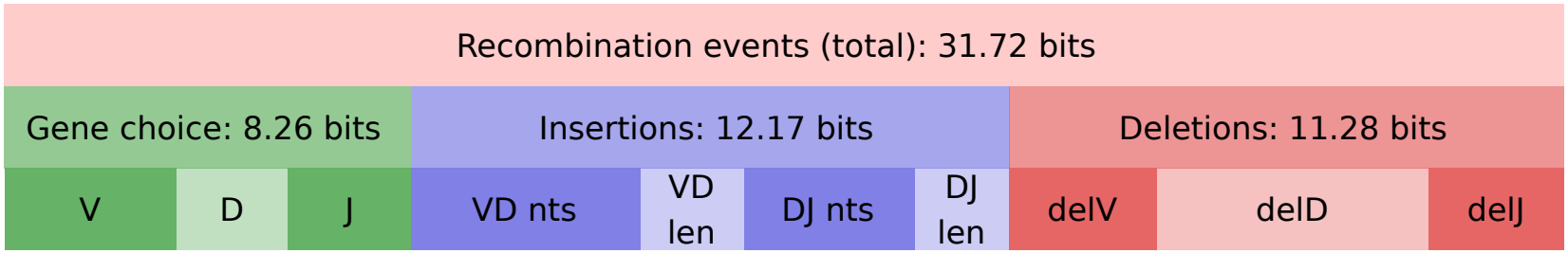


Insertion/deletion in the killifish generative repertoire

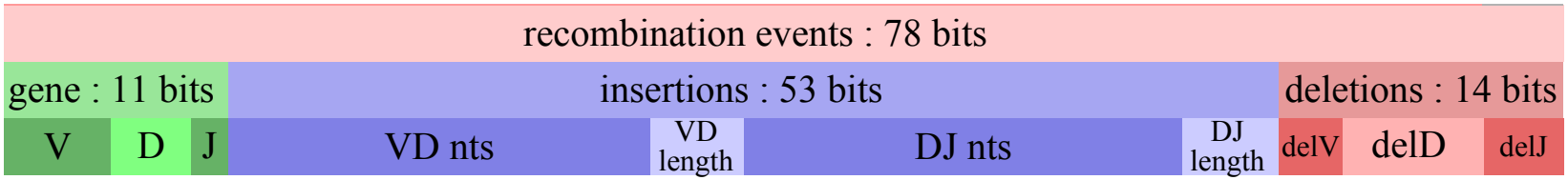


Entropy composition of the killifish generative repertoire

N. furzeri

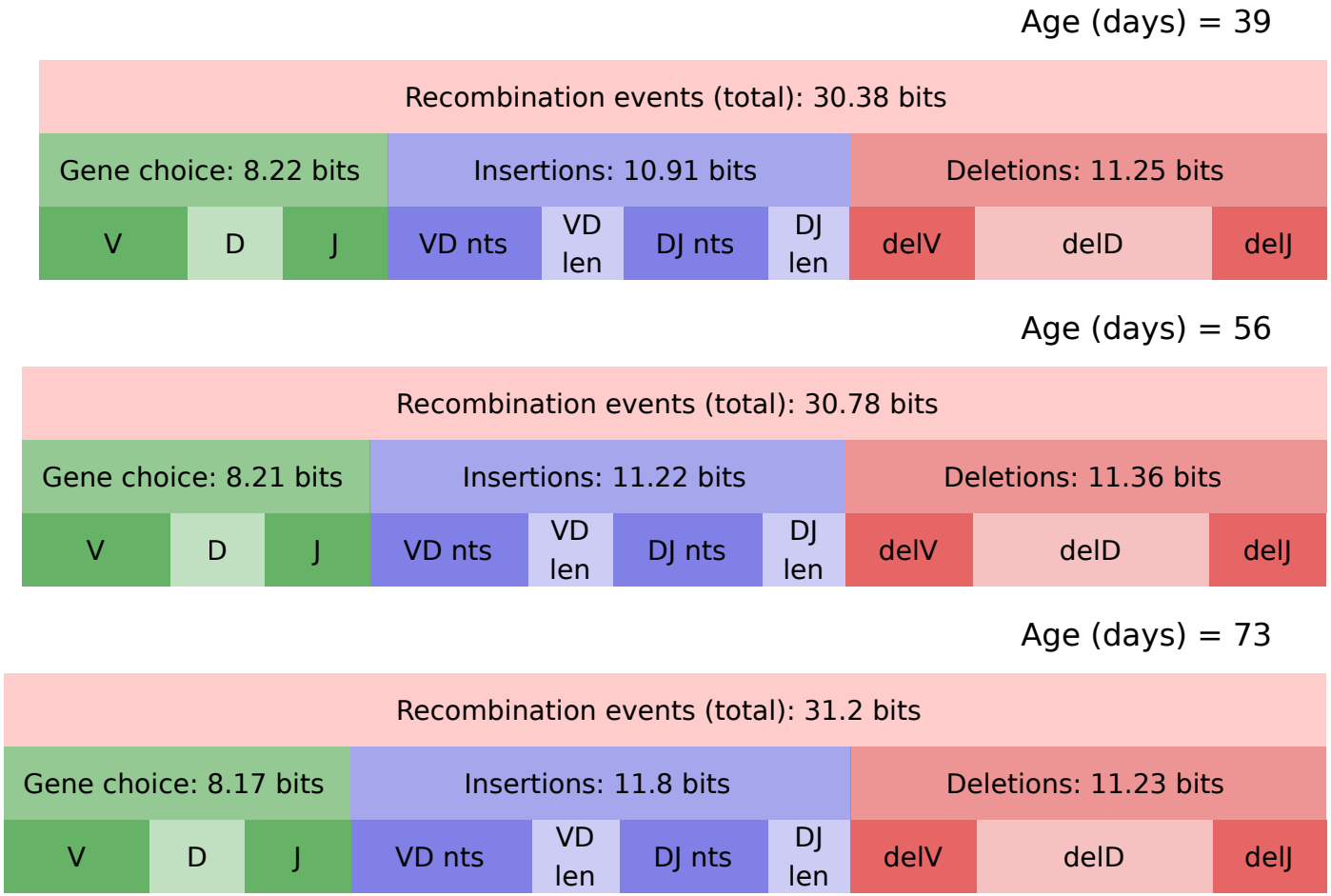


H. sapiens

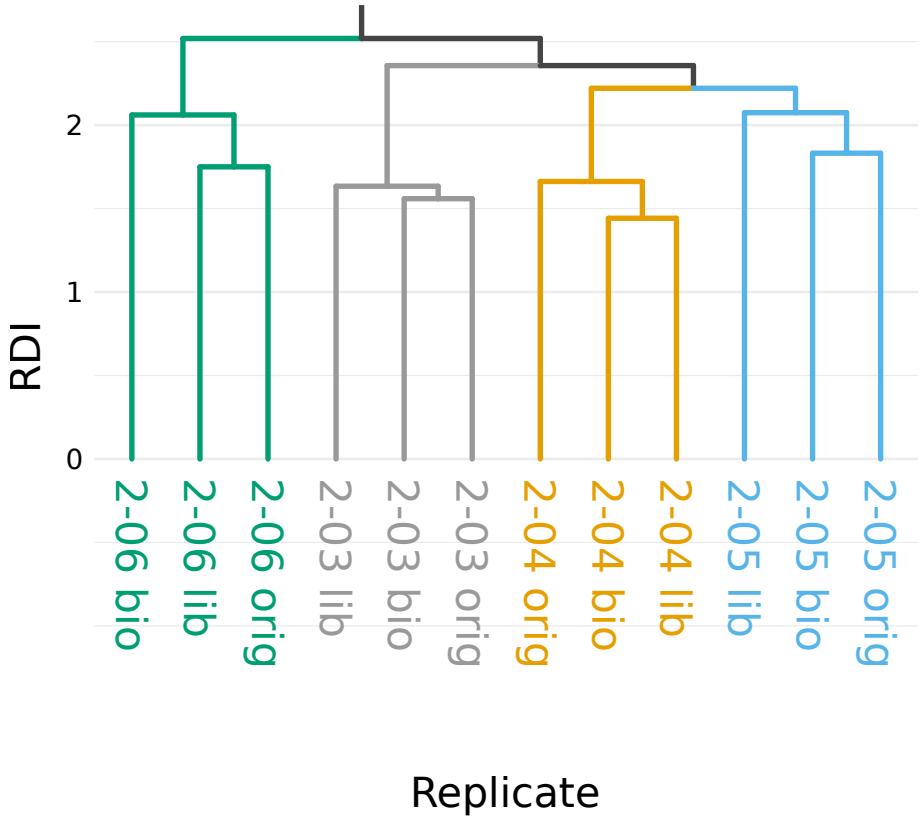


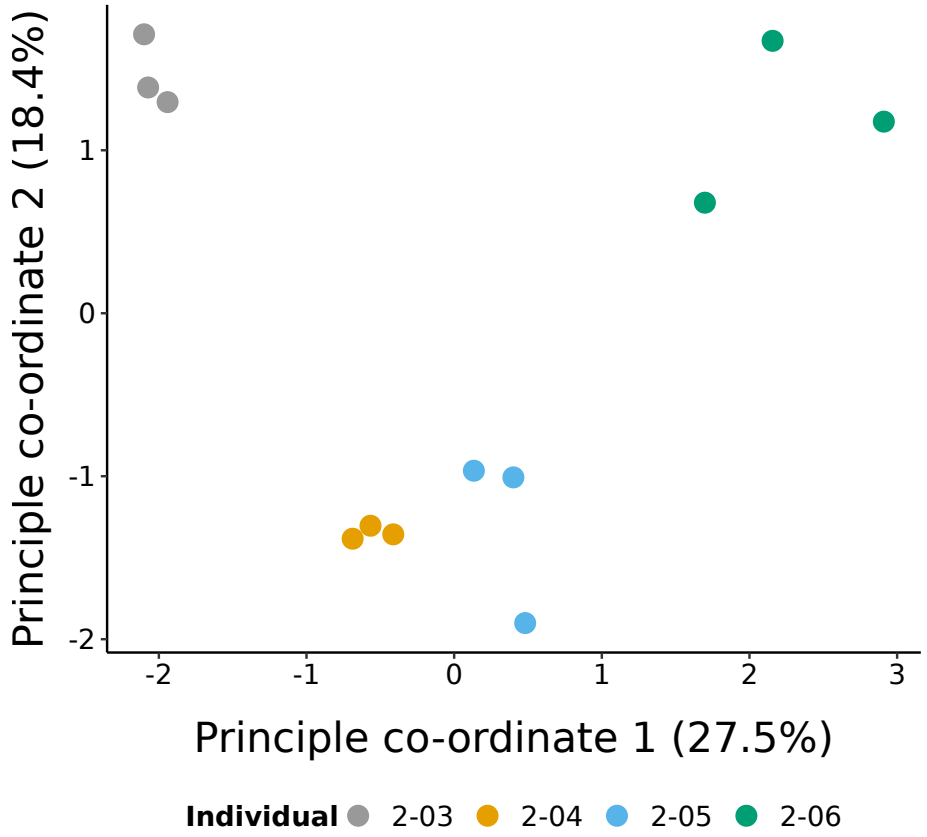
Elhanati et al., Phil. Trans. R. Soc. B (2015)

The generative entropy of the naïve antibody repertoire does not change with age

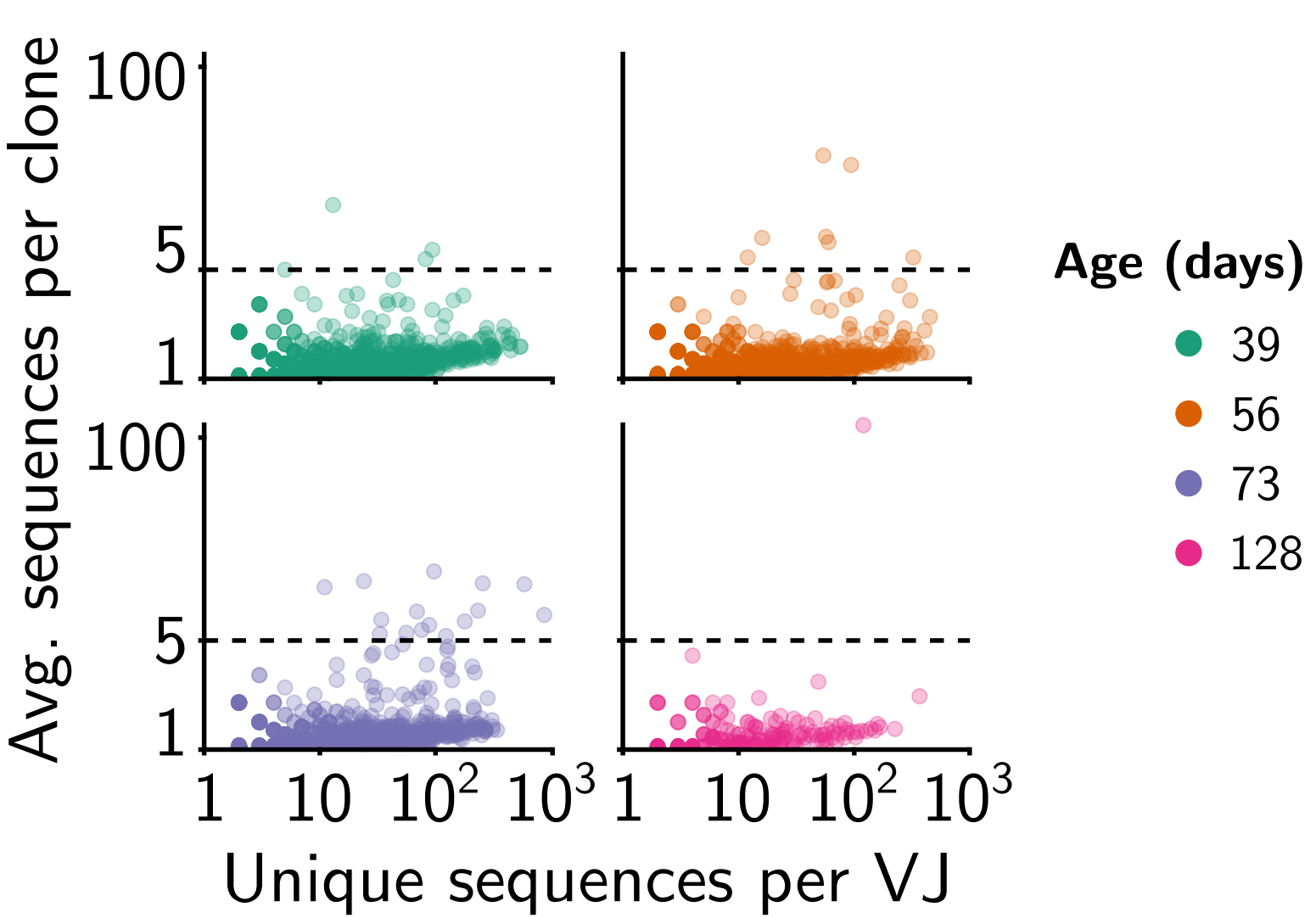


RDI measures can reliably distinguish between individual repertoires

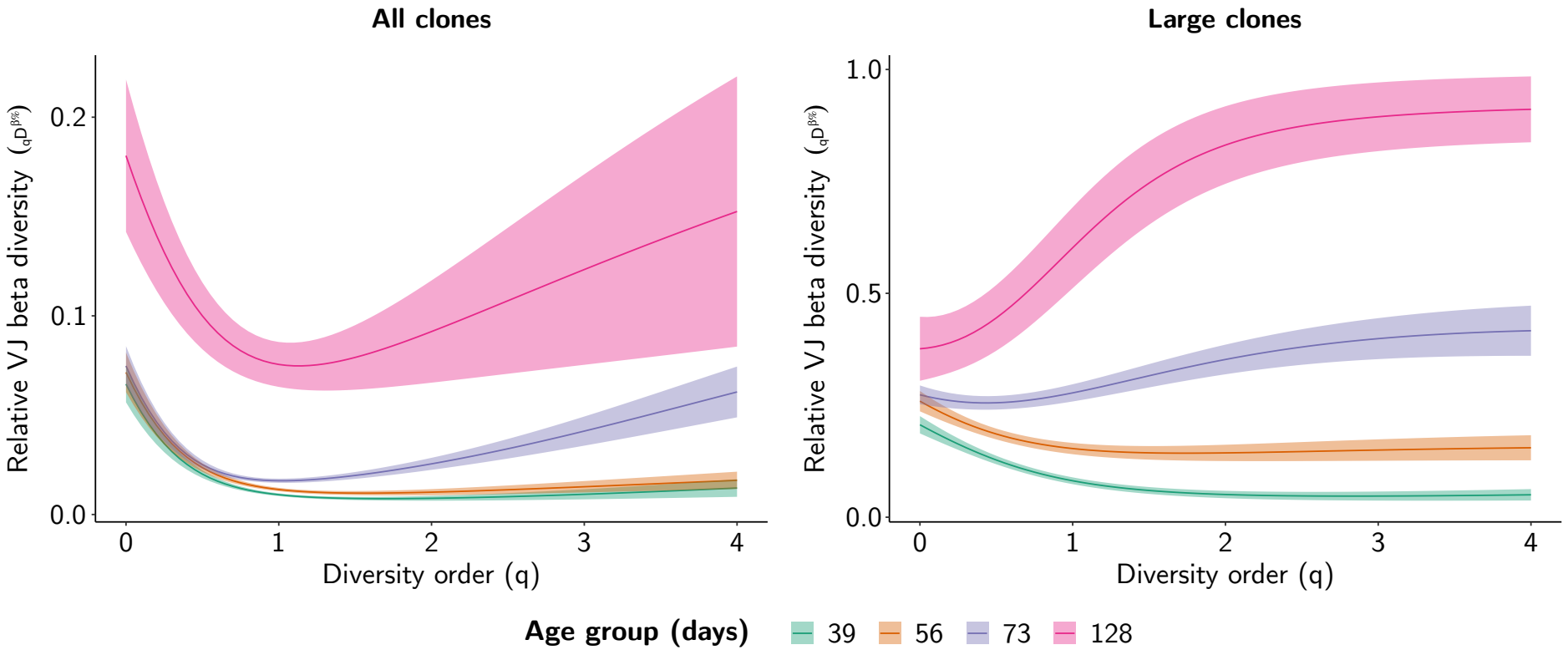




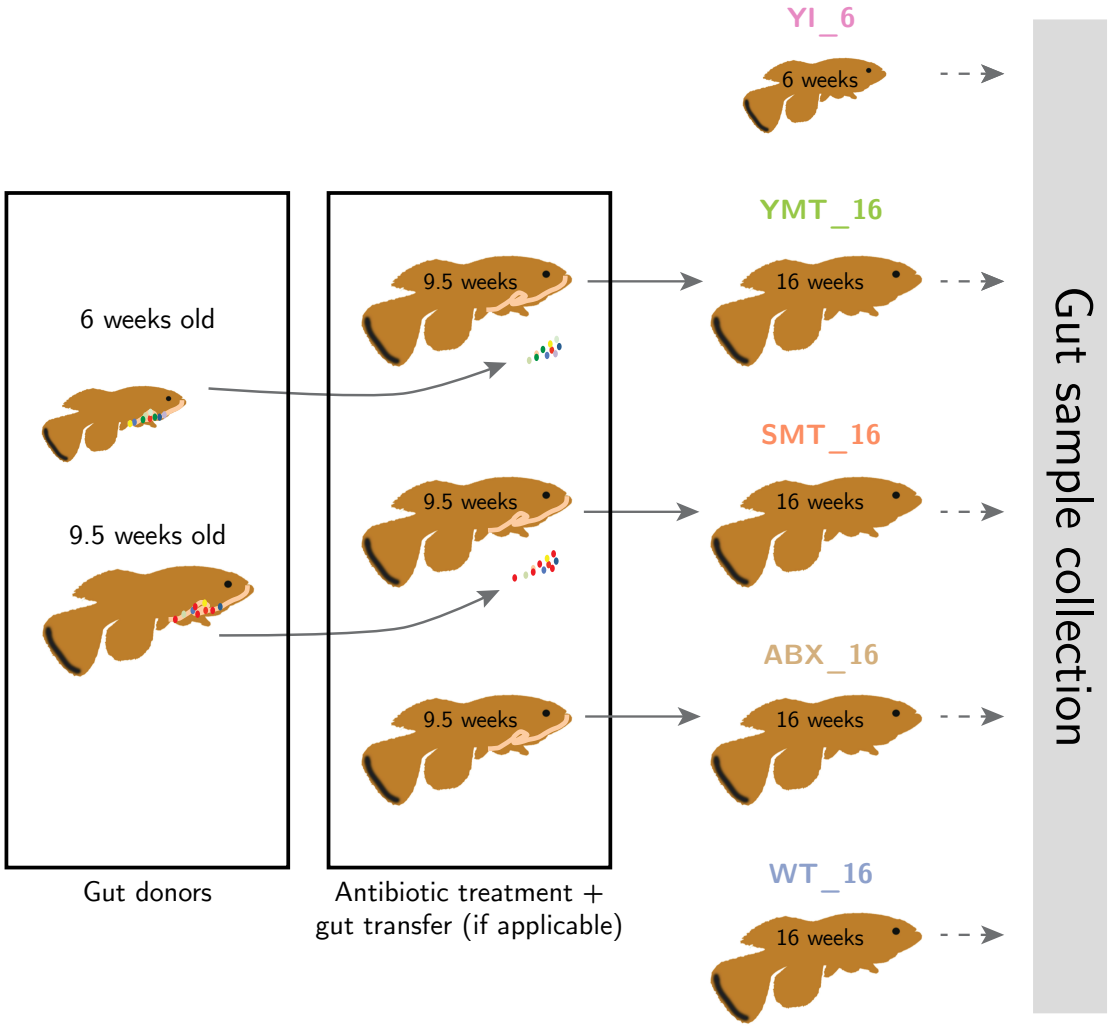
Most VJ combinations are dominated by small clones



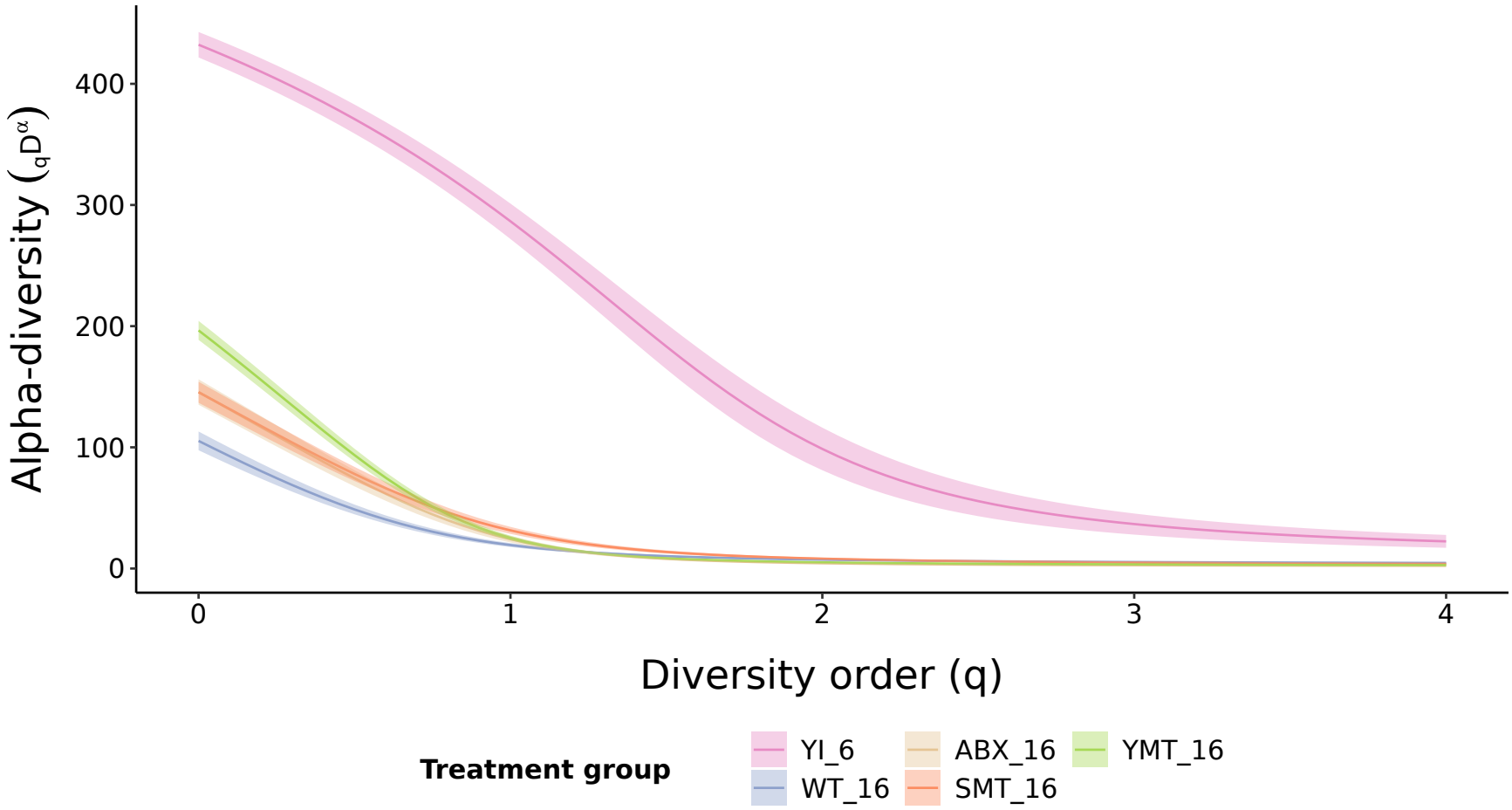
Beta-diversity spectra: ageing cohort



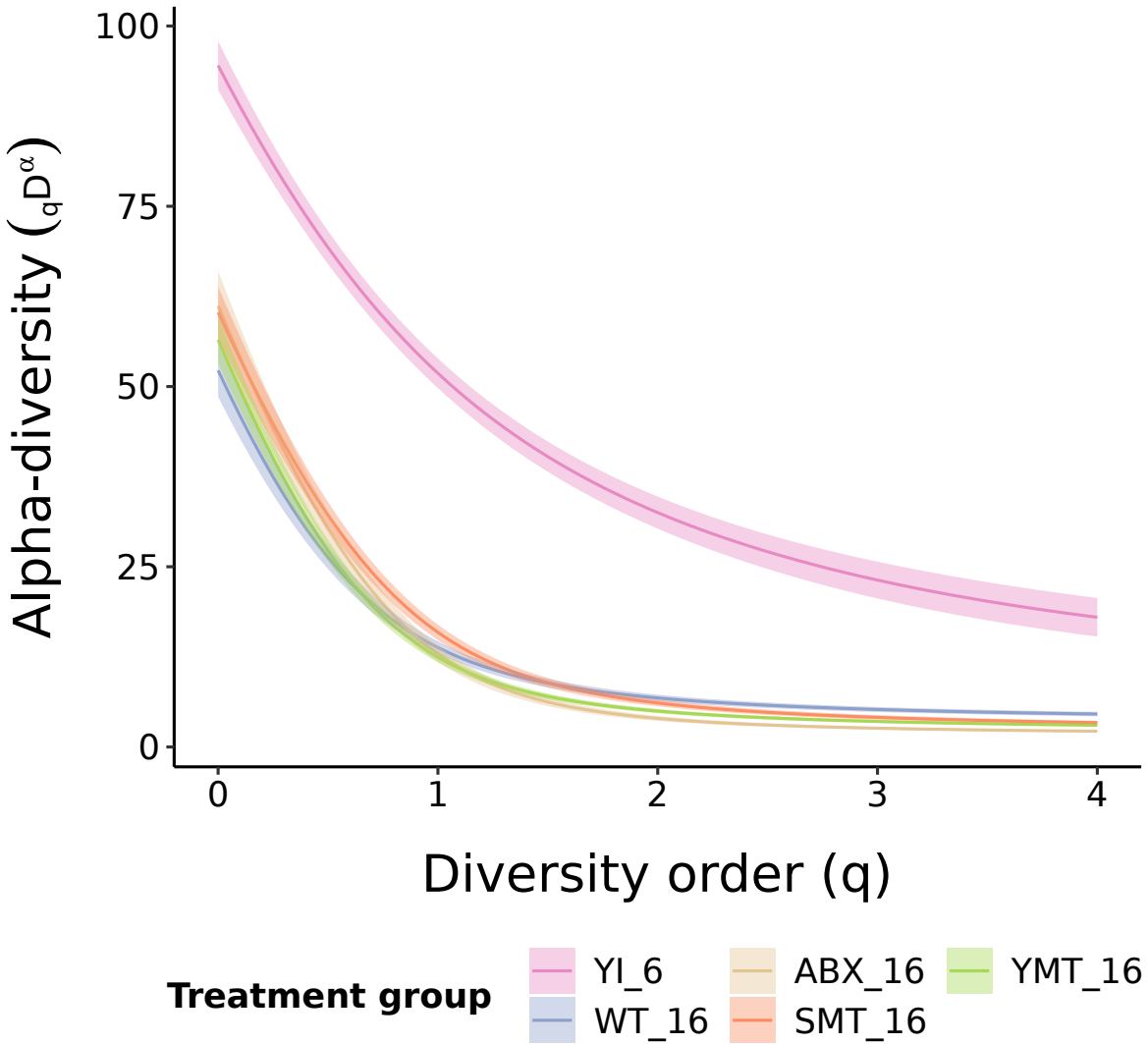
Gut microbiota transfer design



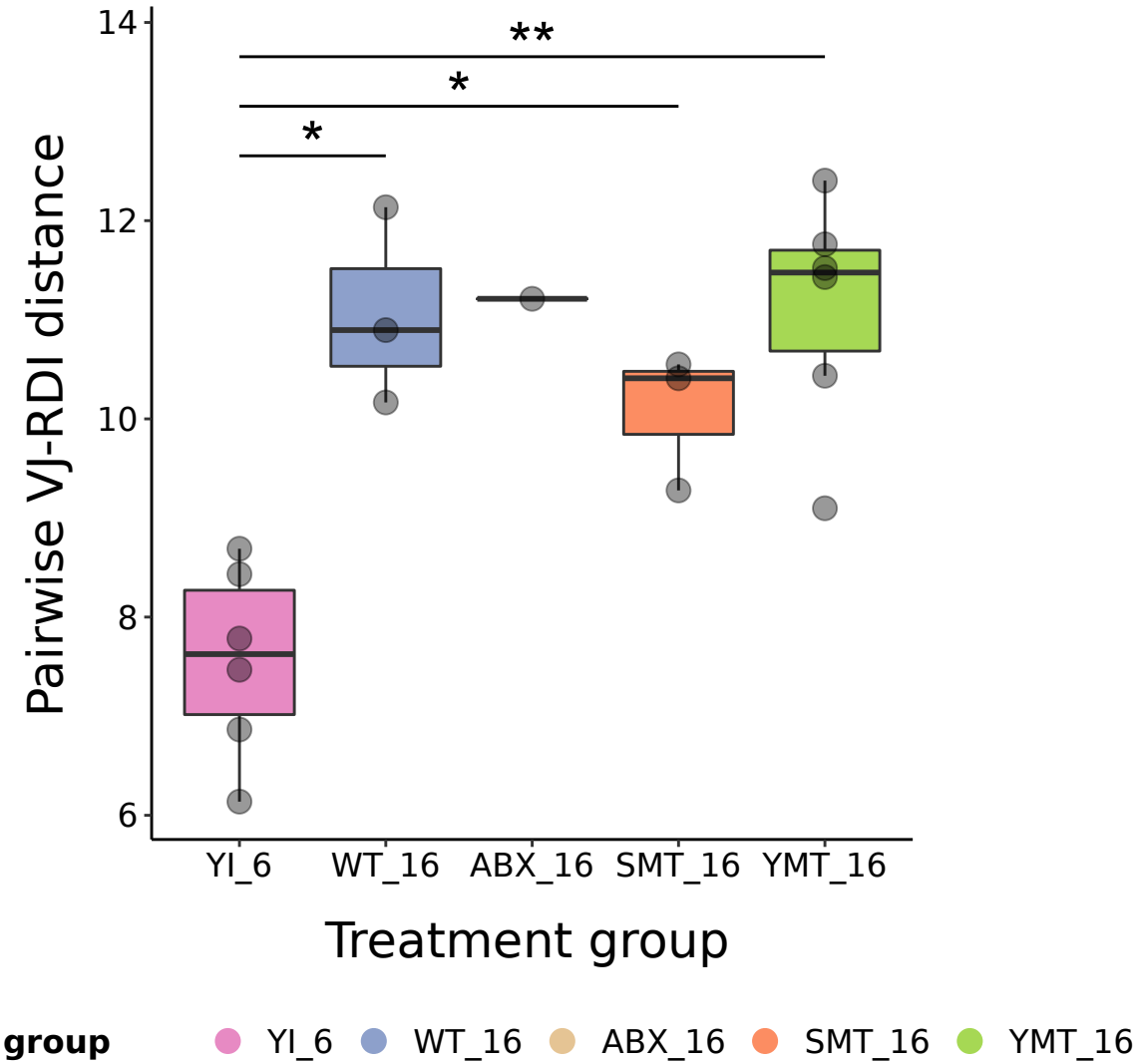
Transfer-group clonal alpha diversity



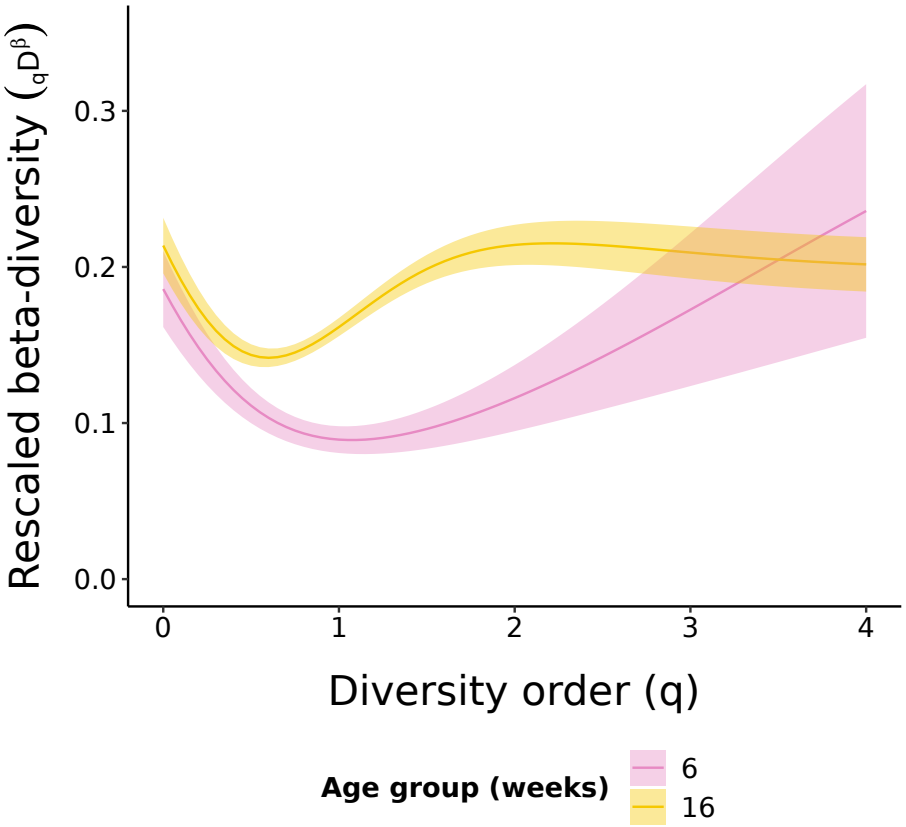
Transfer-group VJ alpha diversity

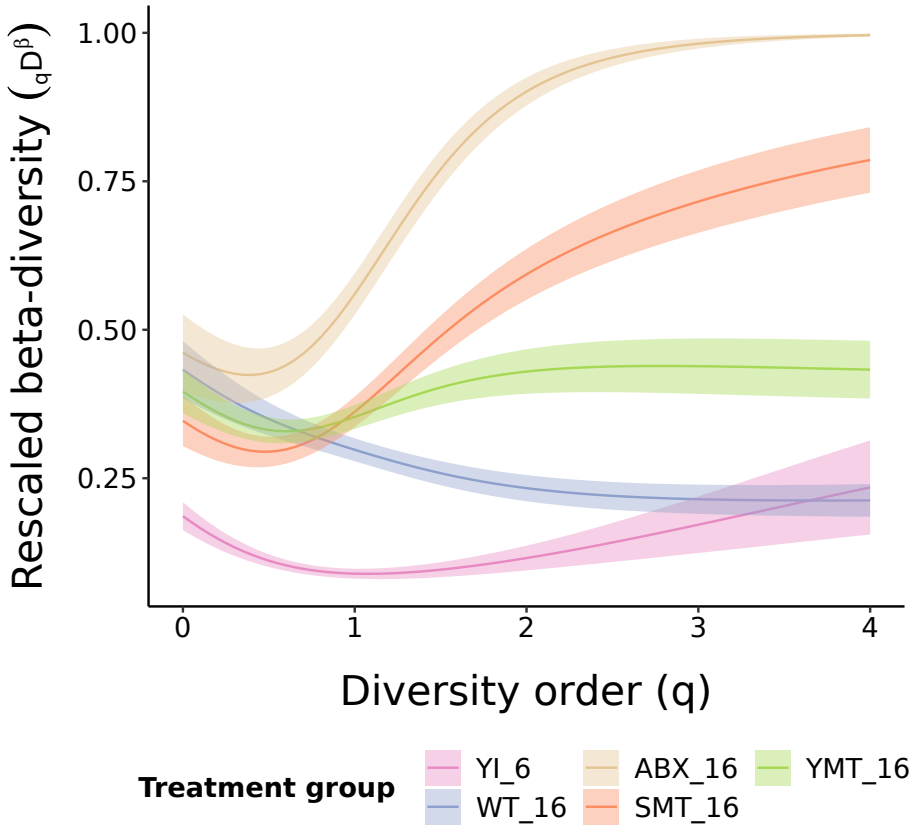


Transfer-group VJ beta diversity

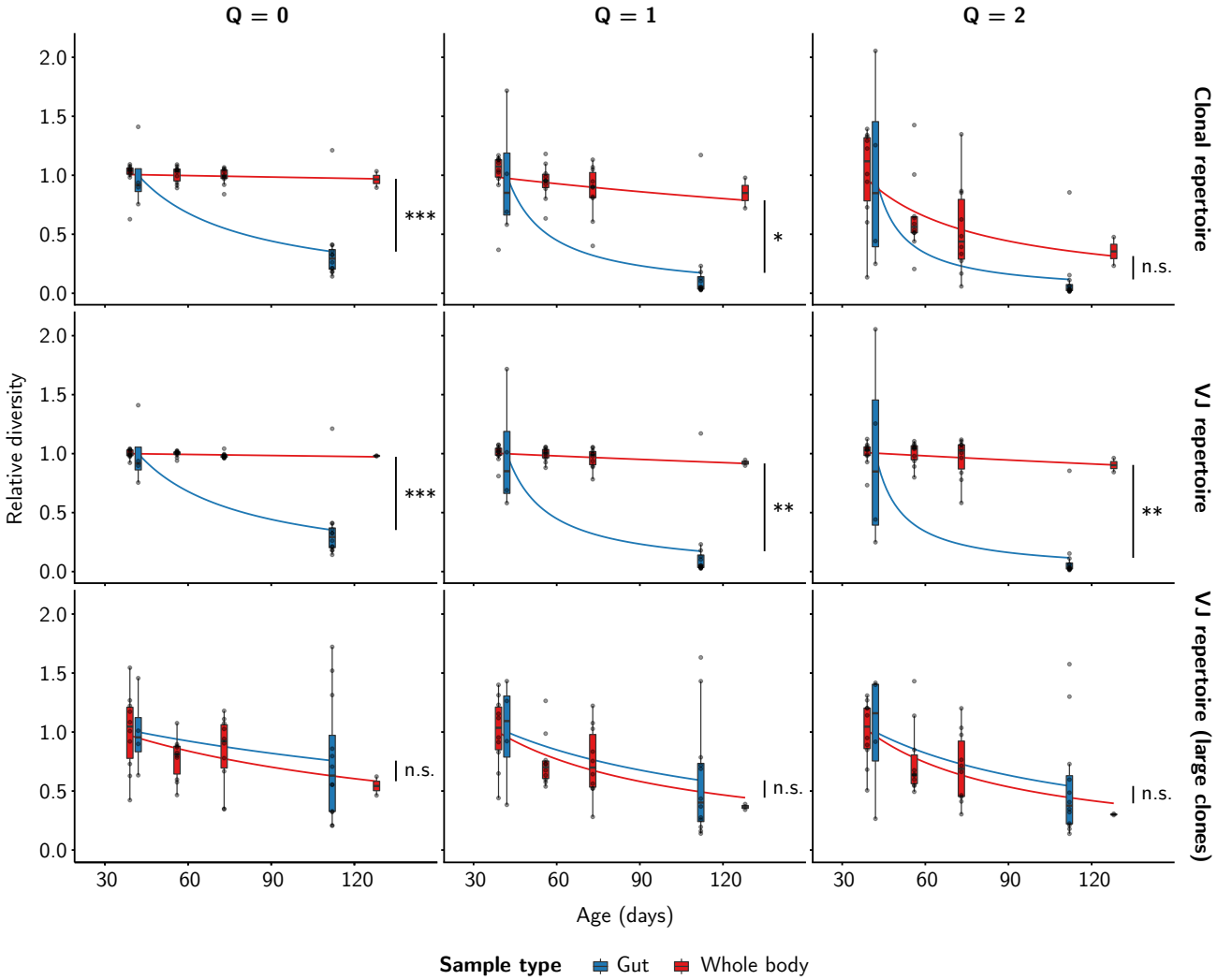


Beta-diversity spectra: gut samples





Relative rate of repertoire diversity loss



Hill diversity for a unitary population X

$$
{}^{q}D(X) = \begin{cases} \left(\sum_{s \in S} p_s^q \right)^{\frac{1}{1-q}} & q \neq 1 \\ \exp\left(-\sum_{s \in S} p_s \cdot \ln p_s \right) & q = 1 \end{cases}
$$

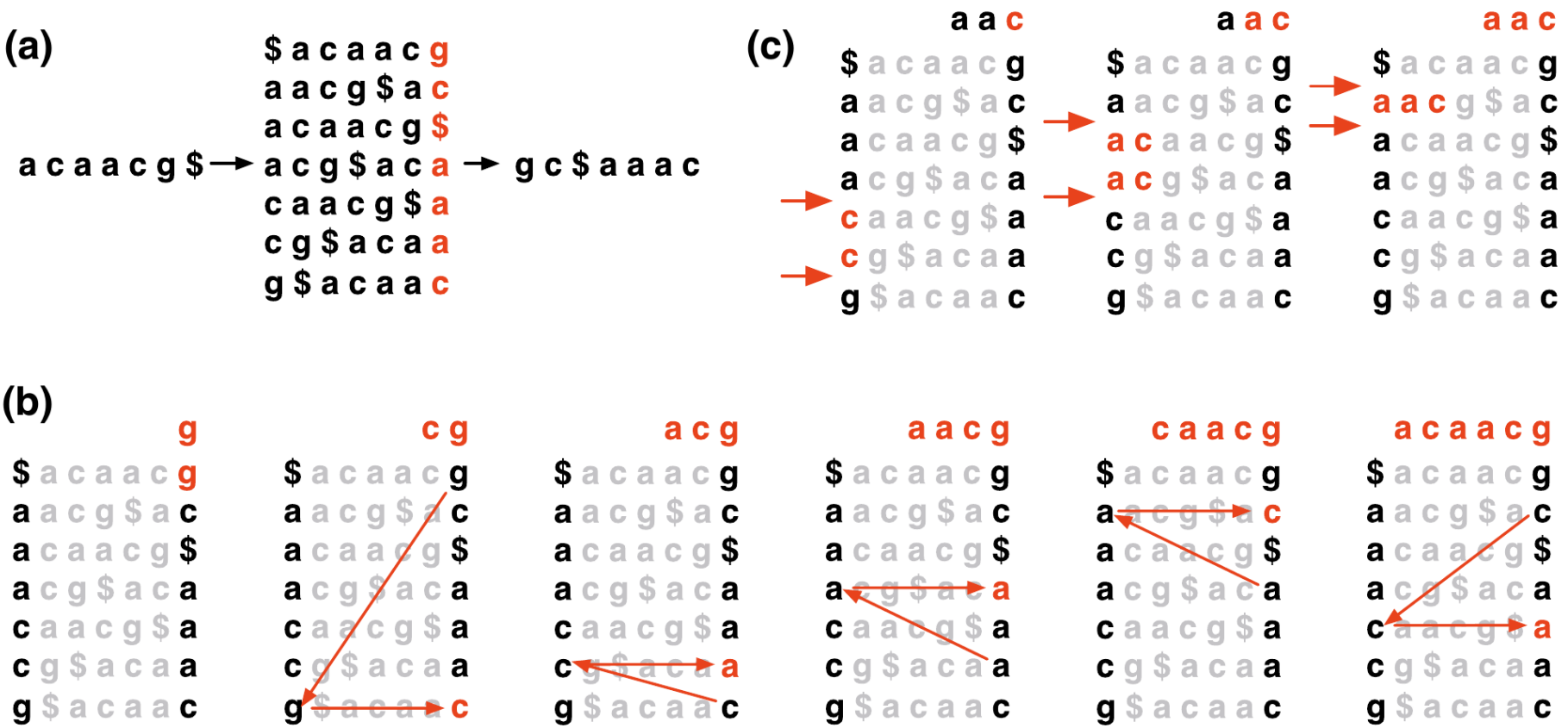
Hill diversity for a structured population C

$$ {}^{q}D_{\gamma}(C) = {}^{q}D(C) $$

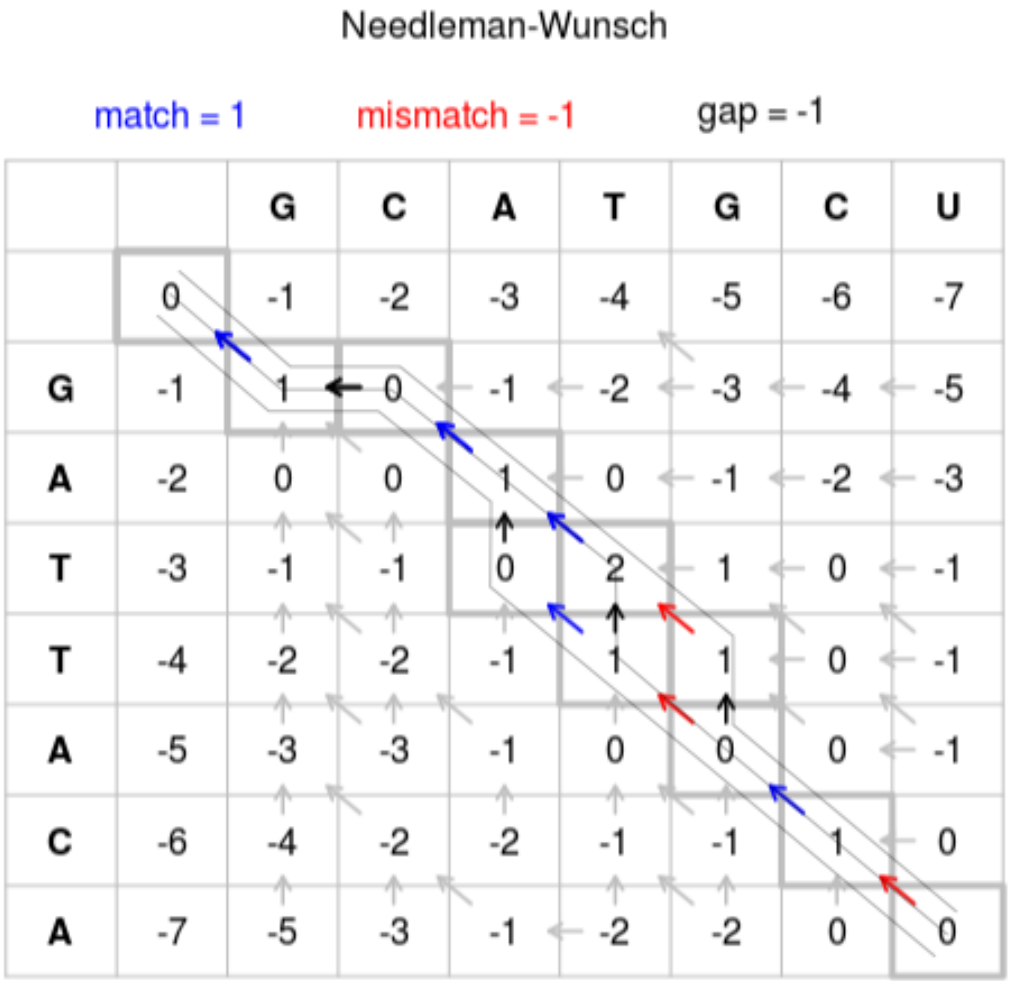
$$ {}^{q}D_{\alpha}(C) = \begin{cases} \left(\frac{1}{M} \sum_{X \in C} [{}^{q}D(X)]^{1-q} \right)^{\frac{1}{1-q}} & q \neq 1 \\ \exp\left(\frac{1}{M} \sum_{X \in C} \ln {}^{1}D(X) \right) & q = 1 \end{cases} $$

$$ {}^{q}D_{\beta}(C) = \frac{{}^{q}D_{\gamma}(C)}{{}^{q}D_{\alpha}(C)} $$

Burrows-Wheeler Transform (BWT)



Needleman-Wunsch Algorithm



		G	C	A	T	G	C	U
	0	-1	-2	-3	-4	-5	-6	-7
G	-1	1	0	-1	-2	-3	-4	-5
A	-2	0	0	1	0	-1	-2	-3
T	-3	-1	-1	0	2	1	0	-1
T	-4	-2	-2	-1	1	1	0	-1
A	-5	-3	-3	-1	0	0	0	-1
C	-6	-4	-2	-2	-1	-1	1	0
A	-7	-5	-3	-1	-2	-2	0	0

Smith-Waterman Algorithm