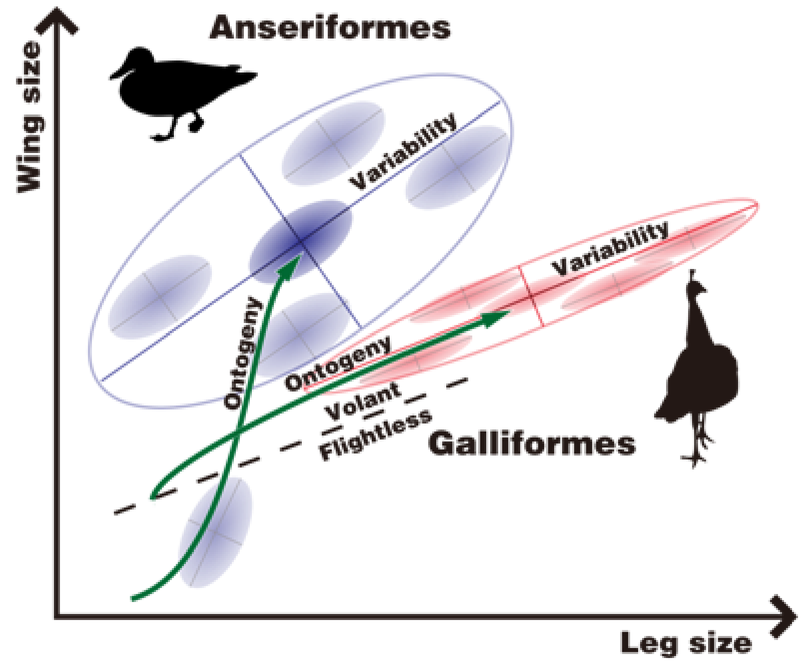
Clade-specific evolutionary diversification along ontogenetic major axes in avian limb skeleton



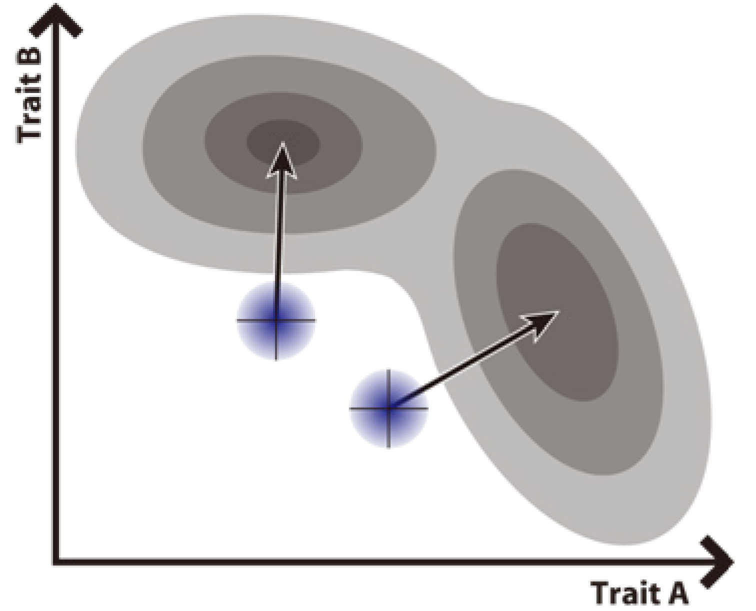
Junya Watanabe
(Kyoto Univ., Japan)

Integration and Evolutionary Bias

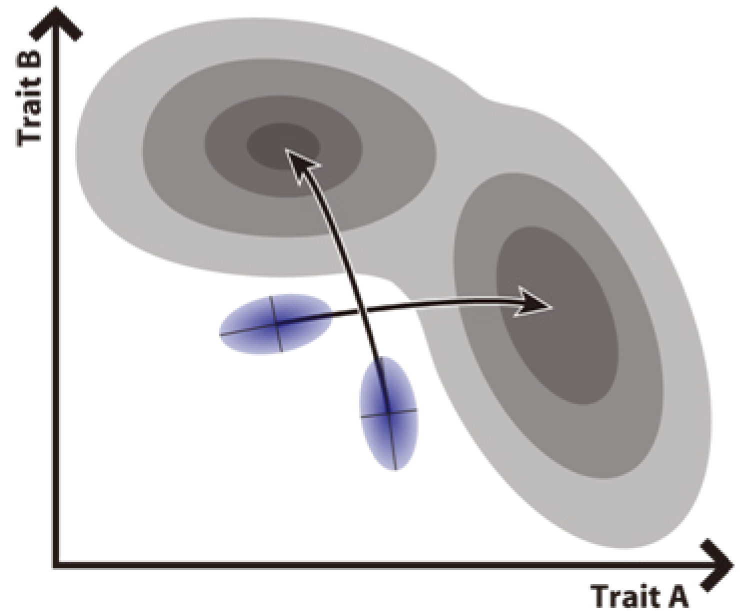
- **Phenotypic integration** can bias evolution
  (e.g., Cheverud, 1982; Steppan et al., 2002; Armbruster et al., 2014; Goswami et al., 2015)

Ex.:

Genetic covariation of traits as genetic lines of least resistance
(Schluter, 1996)



- Different levels of integration:
  - Static
  - Ontogenetic
  - Evolutionary etc.
    (Klingenberg, 2014)



➢ **How are different levels of integration related?**

Integration and Evolutionary Bias

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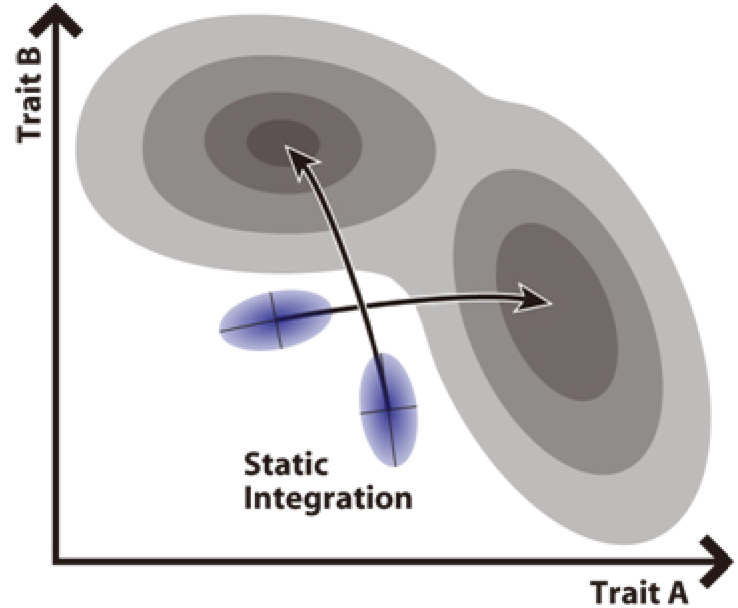

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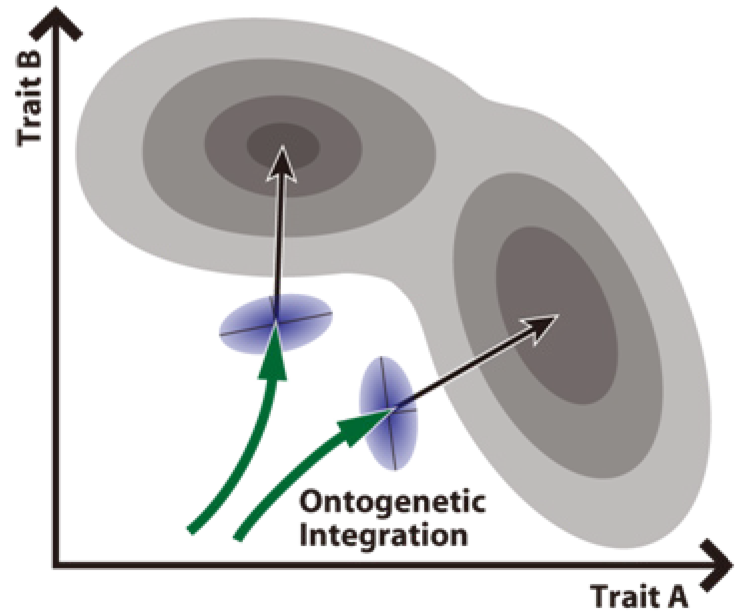
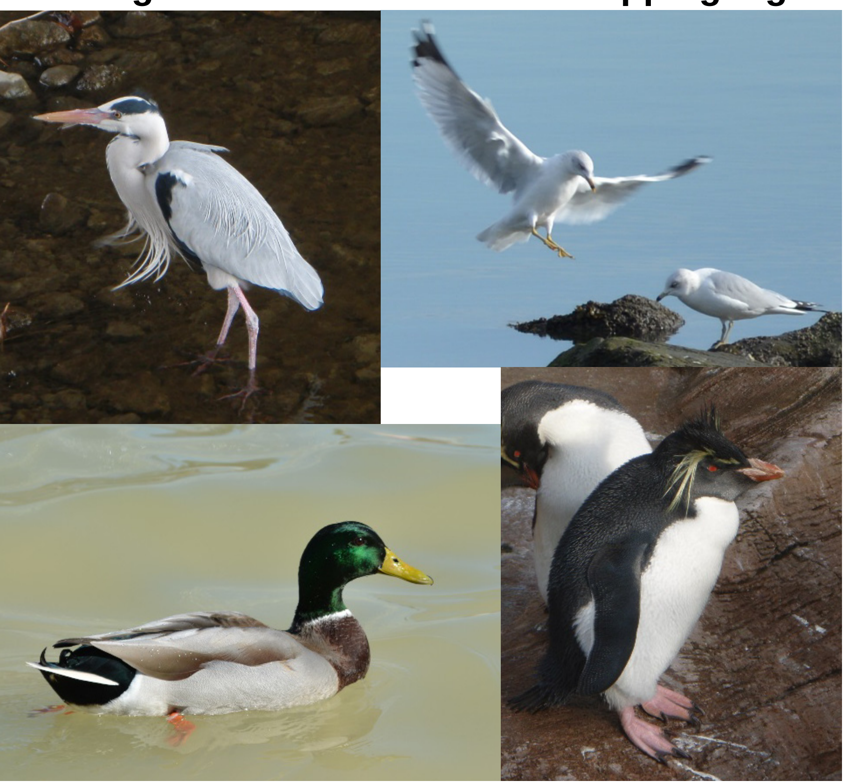


➢ **How are different levels of integration related?**

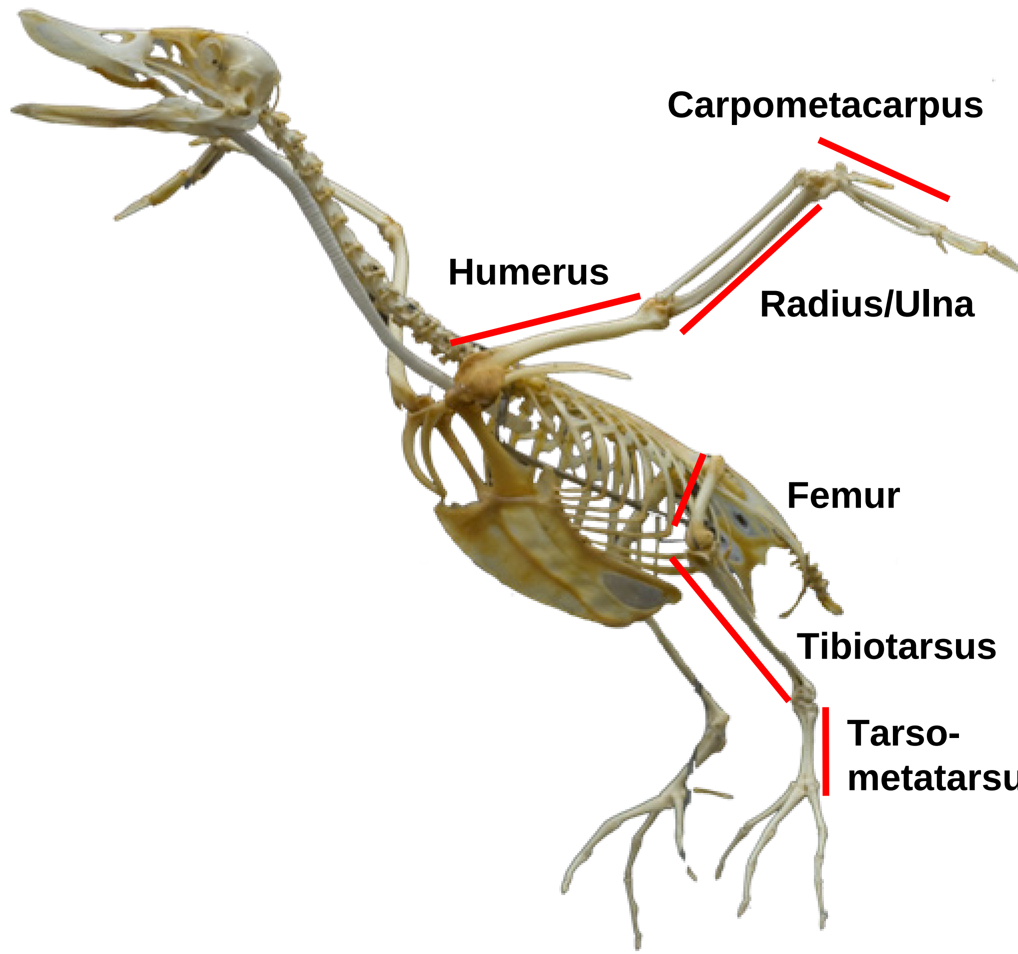
Avian Limb Skeleton

- Enables various locomotion, with suitable proportion
  (e.g., Raikow, 1970, 1985; Storer, 1971; Gatesy & Middleton, 1997; Middleton & Gatesy, 2000)
- Functional signals have been well documented
  (e.g., Zeffer et al., 2003; Nudds et al., 2007; Hinić-Frlog & Motani, 2010; Watanabe, 2017)

Wading **Flapping flight**



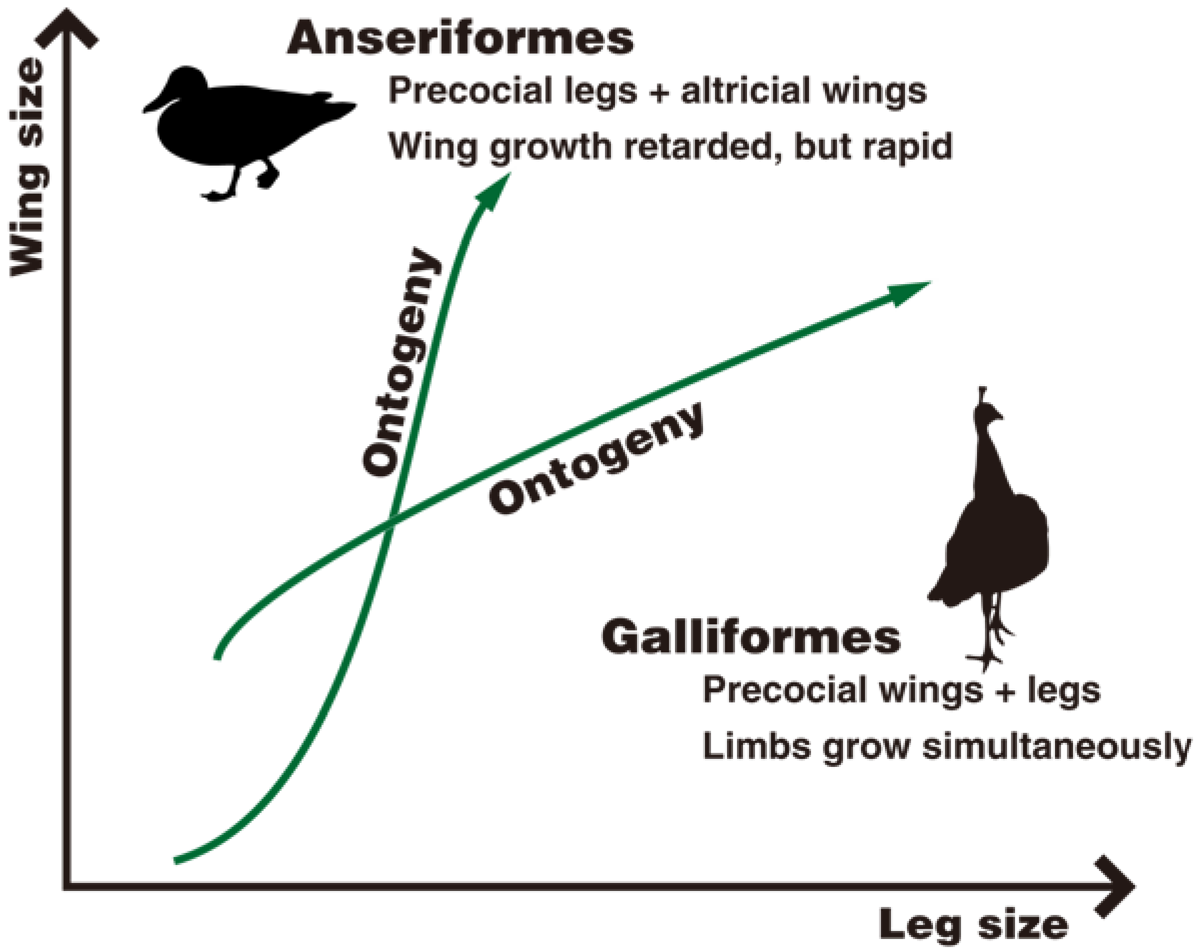
Foot-propelled paddling **Wing-propelled diving**



Ontogeny of Avian Limbs

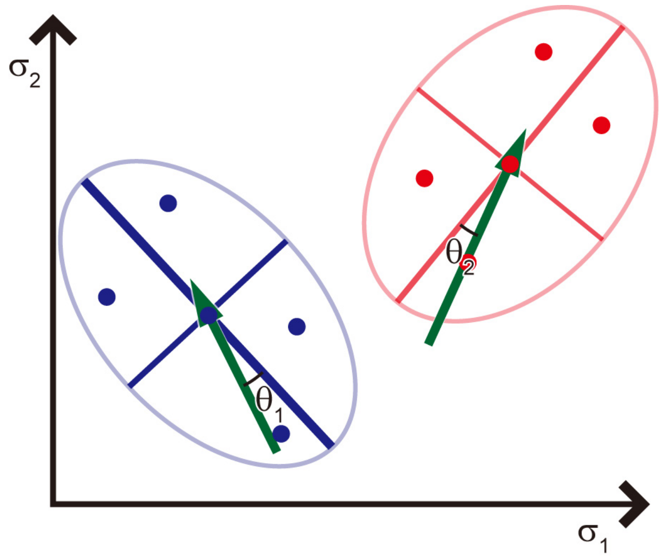
- Highly integrated postnatal ontogeny (Cane, 1993)
- Diverse ontogenetic trajectories among clades (Heers & Dial, 2015)

➢ **Clade-specific ontogeny bias evolutionary variability?**

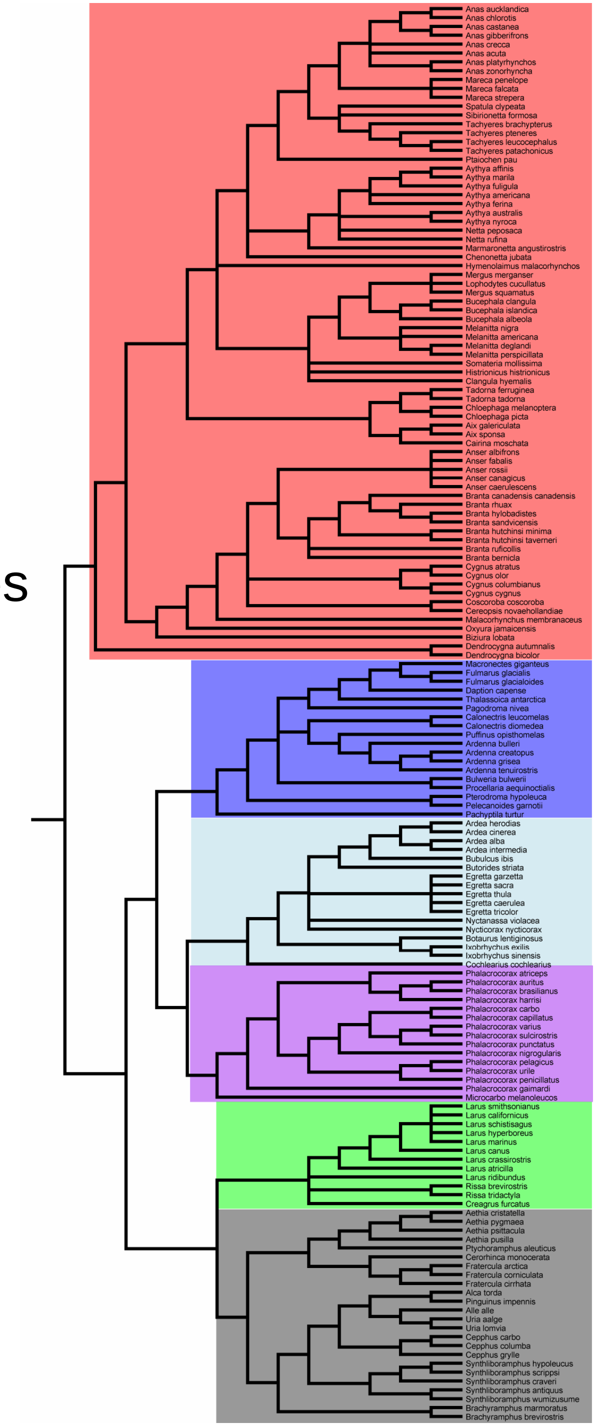


Study Design

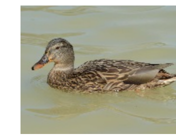
- Compared evolutionary variability and ontogenetic trajectory in 6 avian families
- Ontogenetic trajectory of each family is represented by one selected species
- Included length of 6 limb bones
- Major axes of variation extracted by PCA/pPCA with size-corrected data



Working phylogeny (not drawn to scale; compiled from various sources)



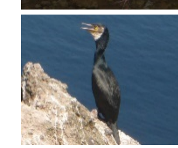
Anatidae



Procellariidae

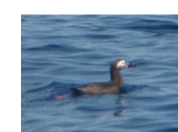
Ardeidae

Phalacrocoracidae



Laridae

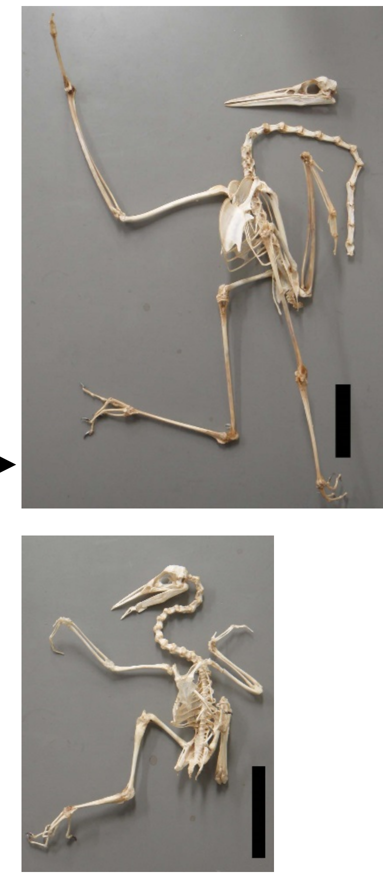
Alcidae



Collection of Ontogenetic Series

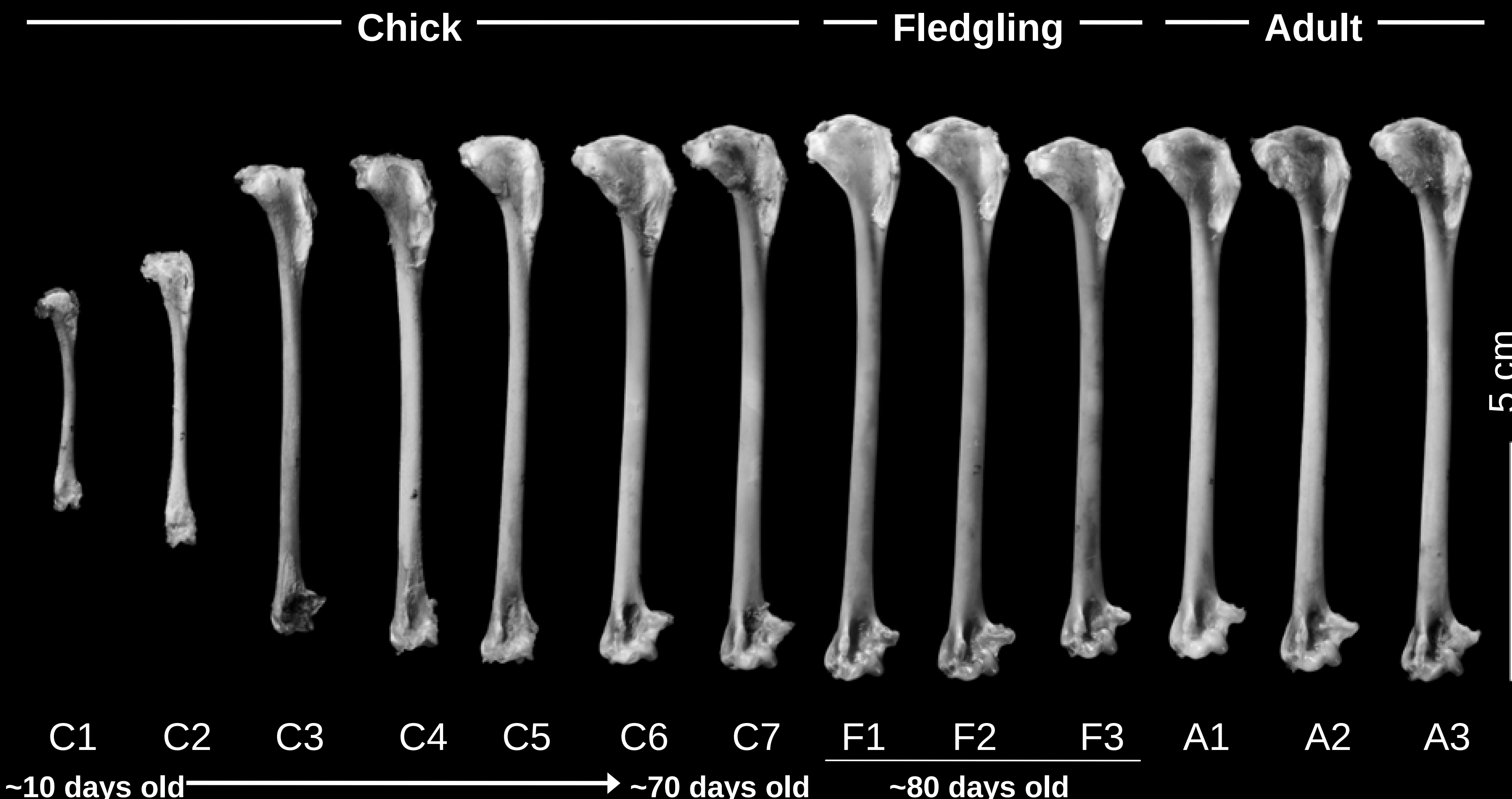
- Conducted fieldworks in breeding colonies
- Prepared series of specimens of known ontogenetic stages

	Chick	Fledgling	Adult
Calonectris leucomelas (Procellariidae)	11	10	7
Ardea cinerea (Ardeidae)	11	2	3
Phalacrocorax capillatus (Phalacrocoracidae)	9	2	2
Larus crassirostris (Laridae)	9		4
Cerorhinca monocerata (Alcidae)	8	4	4



Awashima Island
Calonectris leucomelas

Ontogenetic Series: Humerus, *Calonectris leucomelas*

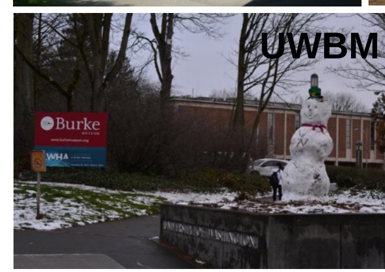


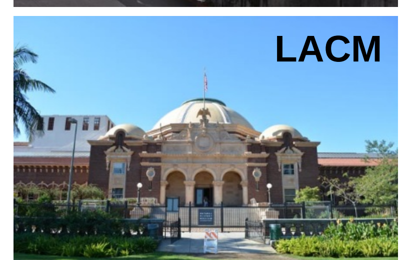
- Limb bones reach their adult size before/around fledging

Data Acquisition

- **Ontogenetic dataset:**
  - Pooled data of chicks + juveniles
  - Data for *Anas platyrhynchos* were taken from the literature (Dial & Carrier, 2012)
- **Evolutionary dataset:** Species means from museum specimens (both modern and fossil, only adults were included):
  - Anatidae 109 spp. (1127 ind.)
  - Procellariidae 25 spp. (344 ind.)
  - Ardeidae 26 spp. (202 ind.)
  - Phalacrocoracidae 17 spp. (298 ind.)
  - Laridae 17 spp. (148 ind.)
  - Alcidae 25 spp. (582 ind.)
- Isometric size was removed before analyses (Burnaby, 1966)

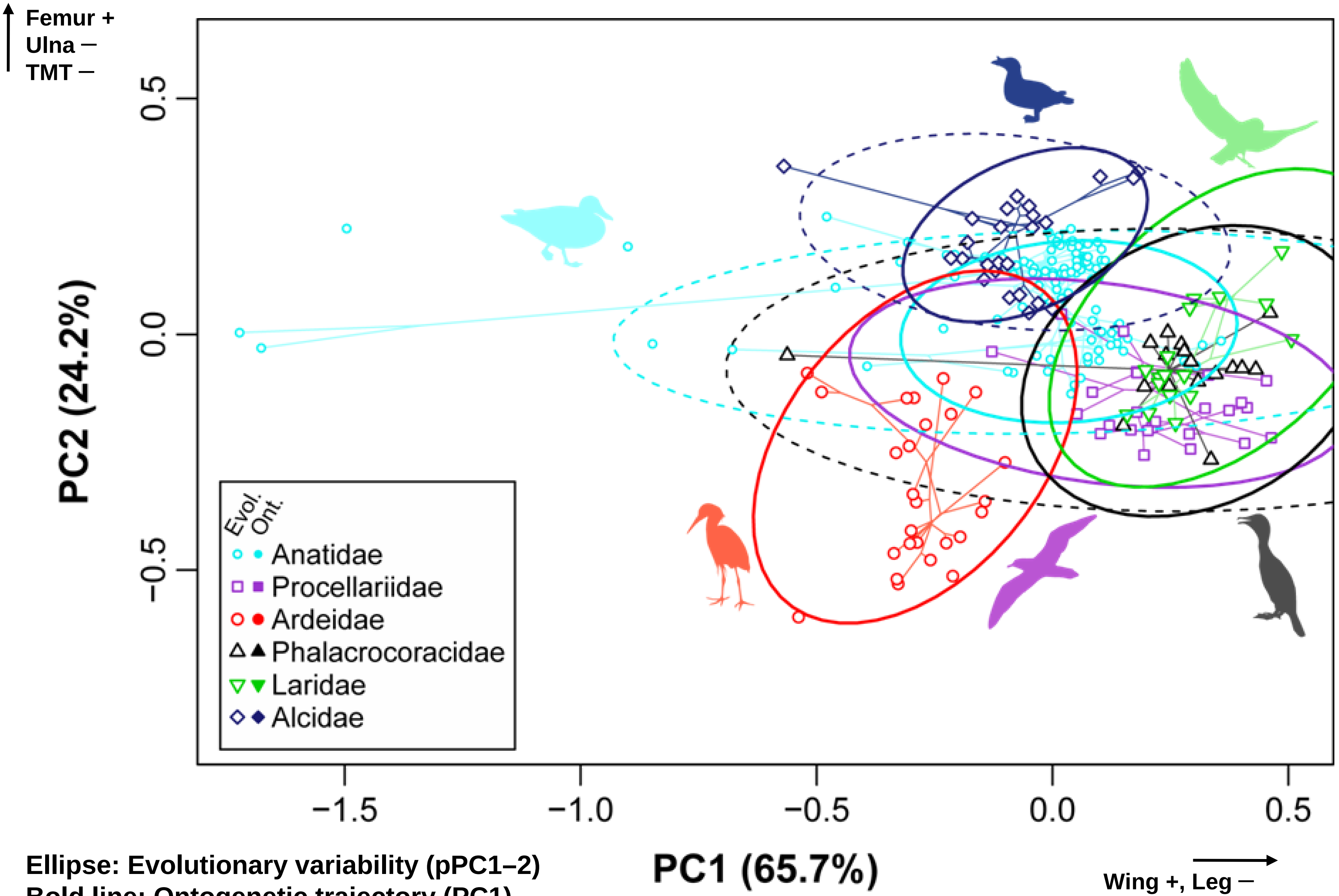






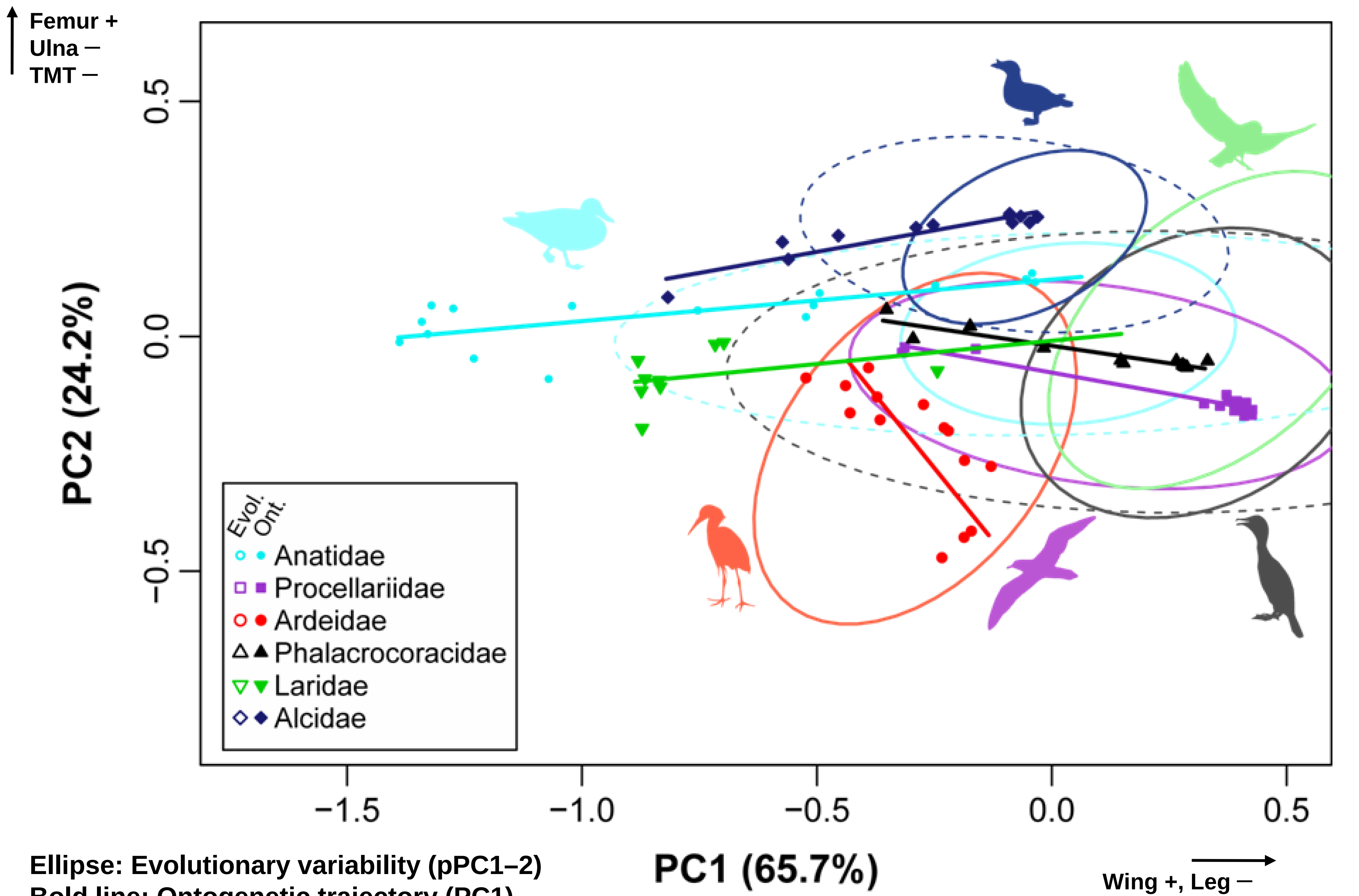
.. and YIO, AMB, LBM, NMNS, HUM, SBMNH, SDMNH, CAS, etc.

Results: Shape Variation Patterns



Ellipse: Evolutionary variability (pPC1–2)
Bold line: Ontogenetic trajectory (PC1)

Results: Shape Variation Patterns



Ellipse: Evolutionary variability (pPC1–2)
Bold line: Ontogenetic trajectory (PC1)

Results: Difference between Ontogenetic PC1s

Upper triangle: *p*-values
(Red: significant difference)

Anatidae	0.00	0.00	0.00	0.01	0.07
0.95	Procellariidae	0.05	0.10	0.09	0.00
0.48	0.71	Ardeidae	0.01	0.01	0.01
0.96	0.98	0.69	Phalacro-coracidae	0.01	0.00
0.96	0.88	0.40	0.93	Laridae	0.00
0.99	0.93	0.45	0.94	0.94	Alcidae

Lower triangle: $\cos\theta$
(Darker blue: more similar)

- Calculated angles between ontogenetic PC1s
- Mean angle: 25.8°
  Range: 8.5°–66.6°
- Tested differences with permutations (10,000 times each), with correction of False Discovery Rate
- Significant differences in most combinations

➢ **Ontogenetic trajectories are diverse among families**

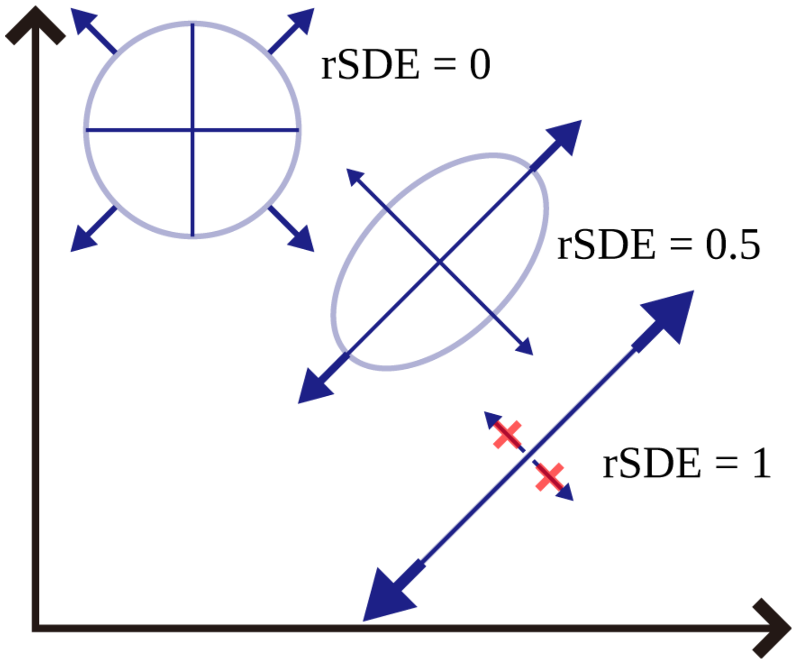
rSDE: Strength of Bias

- Regularized standard deviation of eigenvalues (rSDE):
  - An index of matrix shape (Palvicev et al., 2009; Haber, 2011)
  - Provides a measure of anisotropy of Cov.-matrix
  - Takes a value from 0 (no bias) to 1 (absolute bias)

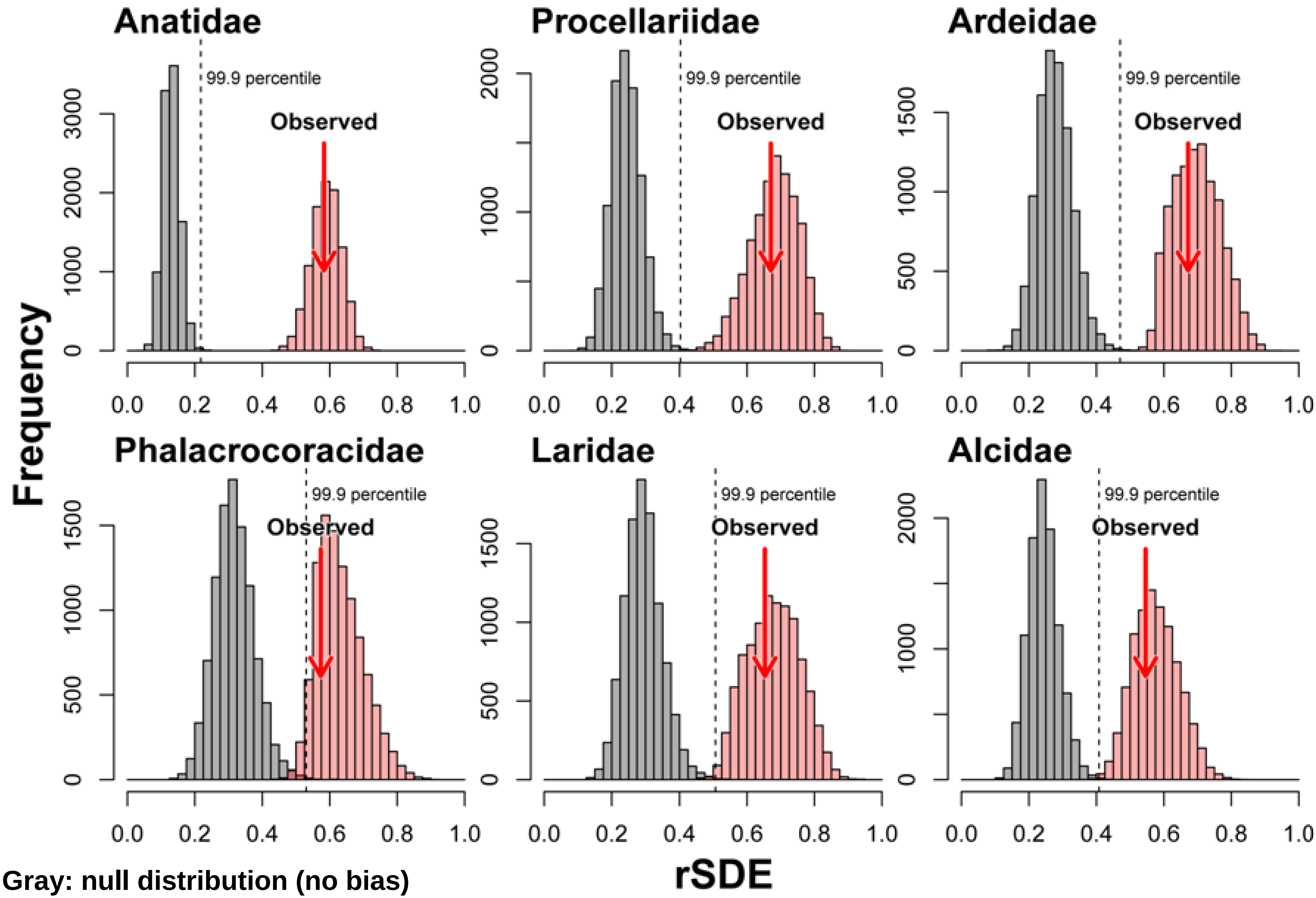
➢ **Observed values were compared with null distributions obtained by simulated BM evolution on working phylos**

$$\mathrm{rSDE} = \sqrt{\frac{p \sum (\lambda - \bar{\lambda})^2}{(p-1) \sum \lambda \sum \lambda}}$$

p: **Number of eigenvalues**
λ: **Eigenvalues**
$\bar{\lambda}$: **Mean of eigenvalues**

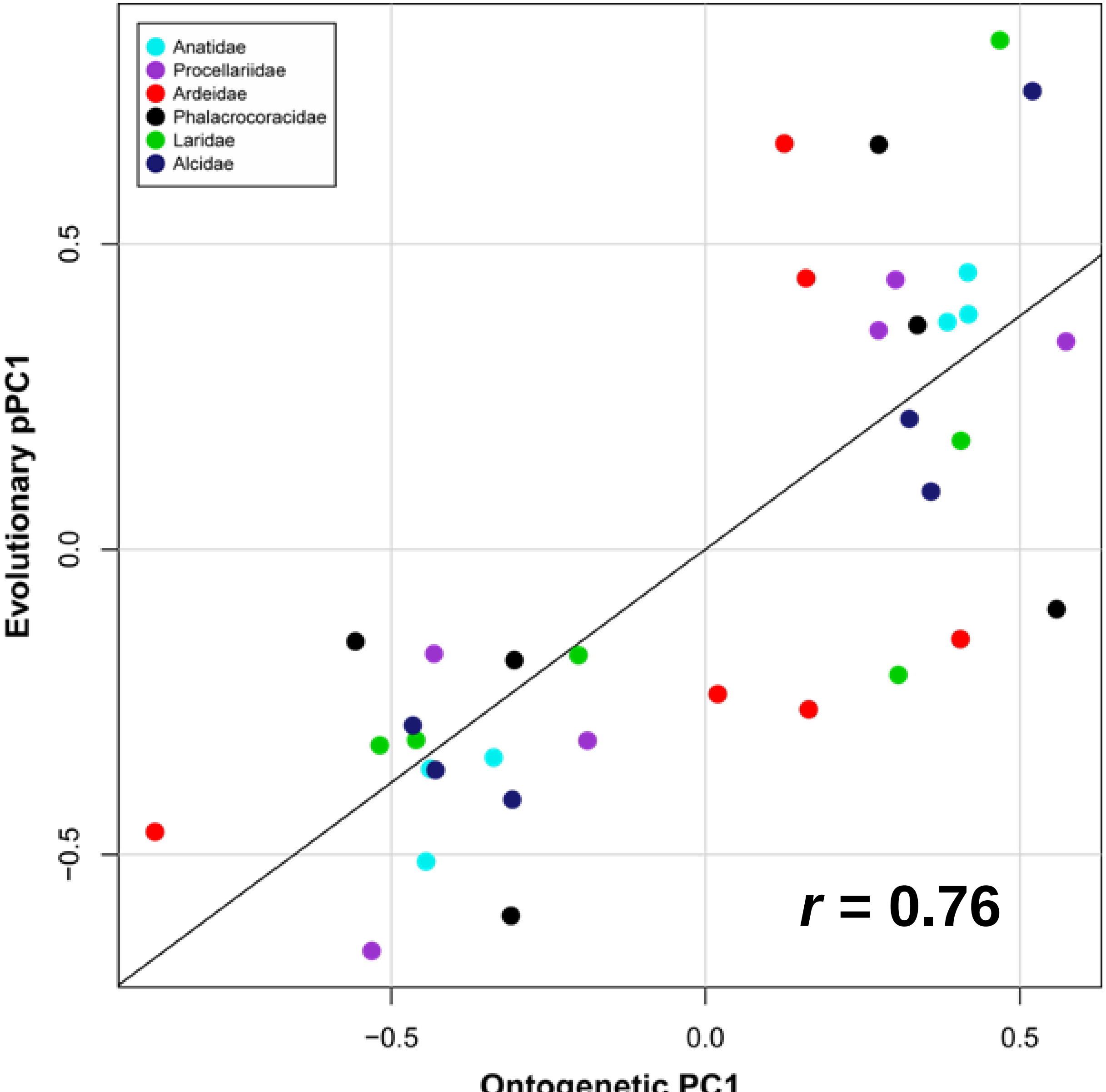


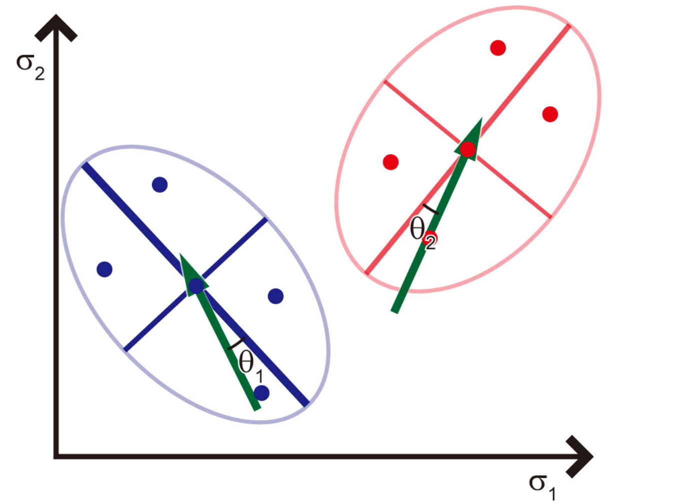
Results: rSDE



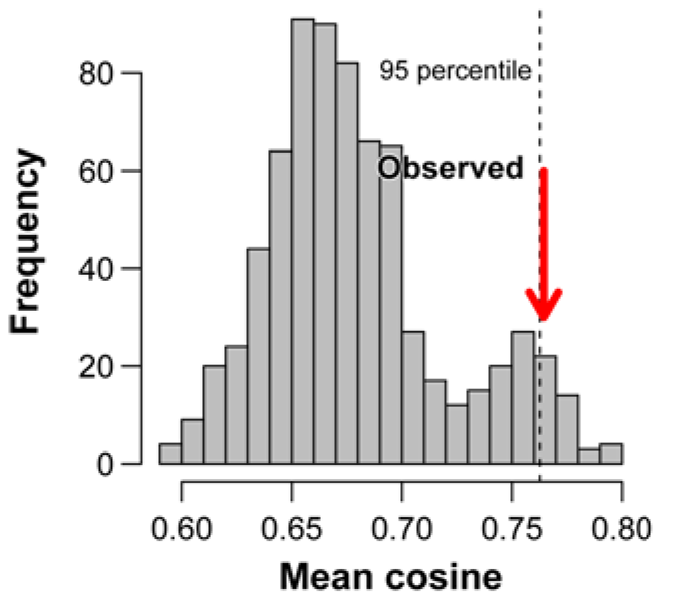
Gray: null distribution (no bias)
Pink: parametric bootstrap

Results: Evolutionary vs. Ontogenetic PC1s





Permutation $p < 0.05$

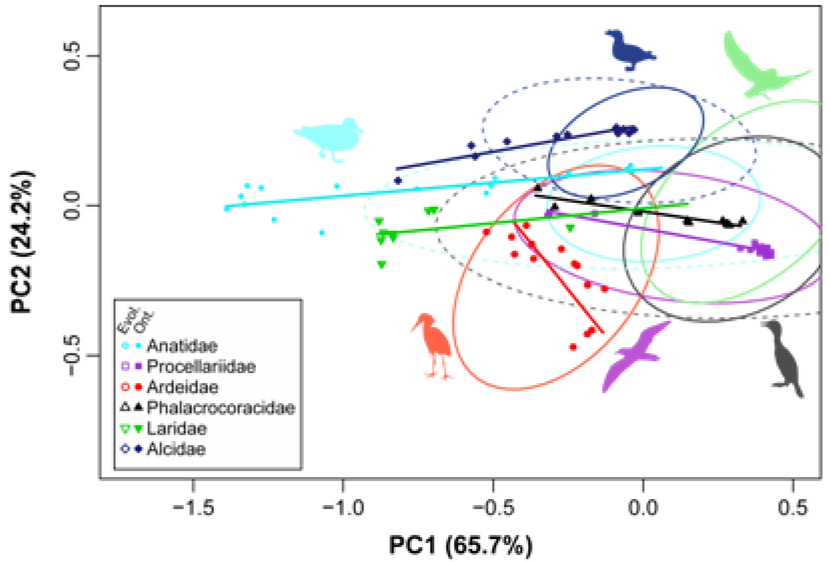


Discussion

- Evolutionary variation is concentrated in the major axis of ontogeny

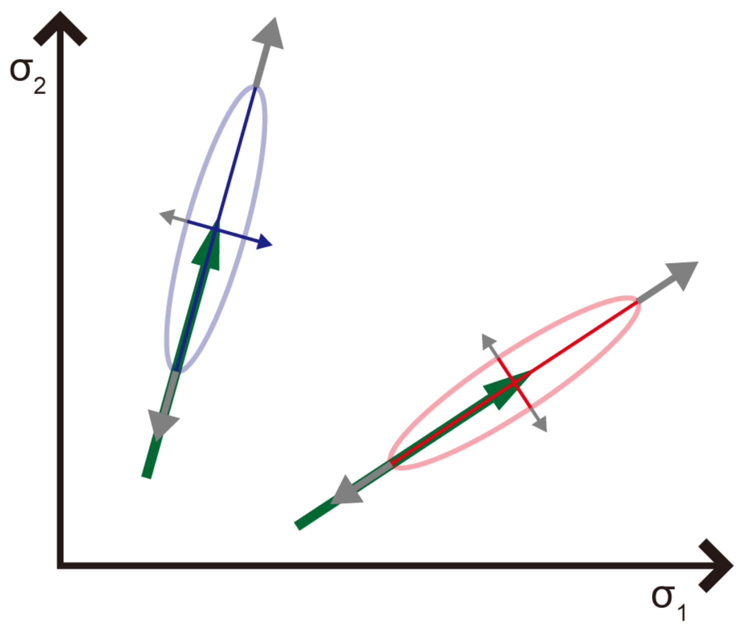
➢ **Bias of evolutionary variability by ontogenetic integration (lines of least resistance)**

➢ **Bias is clade-specific**



- Strong ontogenetic integration of avian skeleton could be a cause

- Main driving forces of divergence (selection/drift) remain elusive at this scale



Implications for Evolutionary Diversification

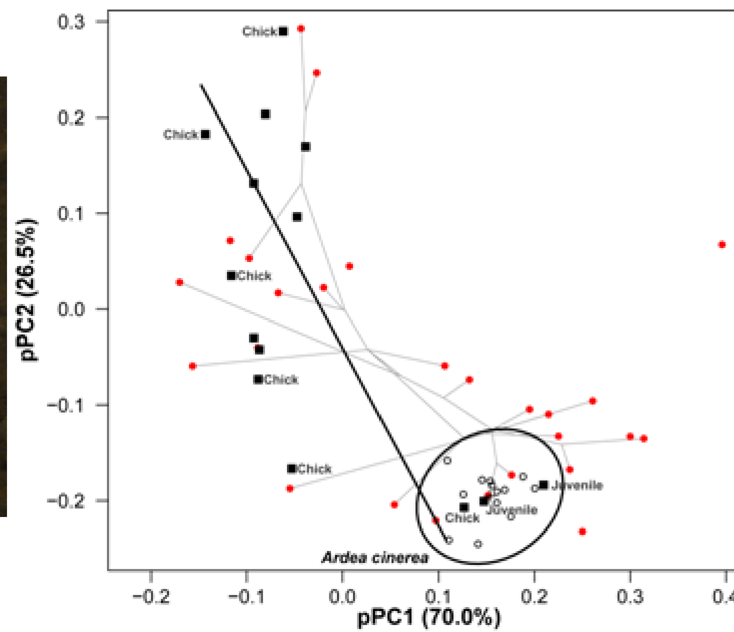
- Differences in ontogenetic integration patterns might explain clade-specific patterns of evolutionary diversification

Ex.:

- **Diversity of leg length in Ardeidae**
  In Ardeidae, leg length corresponds to main foraging habitat
  Highly variable even among close relatives (Boev, 1988, 1989)

➢ **Characteristic ontogeny of the family may have facilitated the diversification**

Foraging in deeper streams



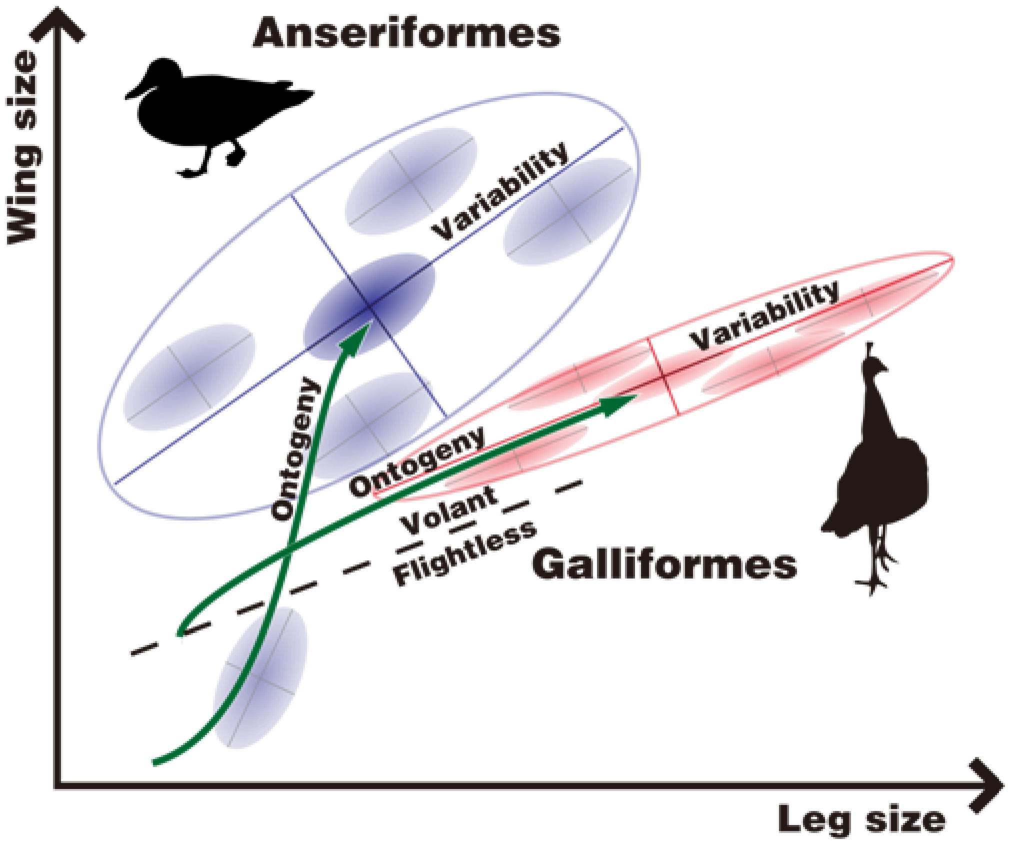
Implications for Evolutionary Diversification

Murray & Vickers-Rich (2004):

Clade-specific propensity for flightlessness might result from differences in ontogenetic trajectories?

This study:

- Clade-specific ontogenetic trajectory may bias evolutionary variability
- **Ontogenetic basis for flightlessness**



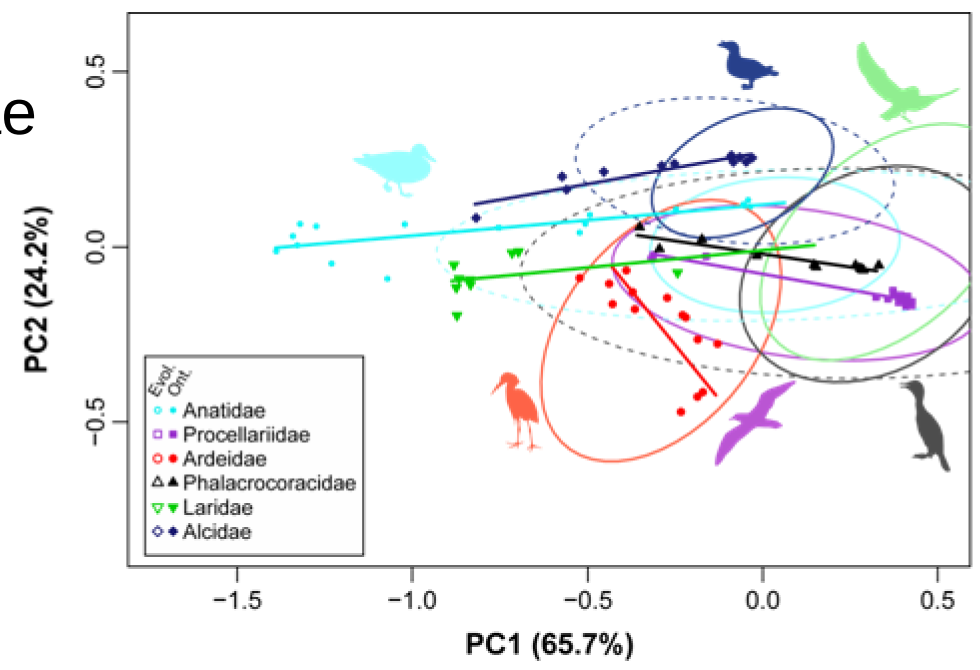
Summary

- Relationship between ontogenetic trajectory and evolutionary variability was examined in 6 families
- **Clade-specific ontogenetic trajectories seem to bias evolutionary variability**

➢ Such bias might explain differences in evolutionary diversification patterns in avian clades

Ex:

Long-leggedenss in Ardeidae
Flightlessness in Anatidae



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Size-correction

- Variation patterns in the shape space is examined
- Log-transformed variables were projected onto the shape hyperplane perpendicular to the isometric size axis with Burnaby's (1966) method

$$\mathbf{X}' = \mathbf{X}(\mathbf{I} - \boldsymbol{\mu}\boldsymbol{\mu}^T)$$
$$\boldsymbol{\mu} = p^{-1/2}\mathbf{1}$$

$\mathbf{X}$: data matrix
$\mathbf{X}'$: shape data matrix
$\mathbf{I}$: $p \times p$ identical matrix
$\boldsymbol{\mu}$: size vector
p: number of variables
$\mathbf{1}$: $p \times 1$ vector with 1