Parassitismo e interazione tra specie alloctone e autoctone: il caso degli Sciuridi

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Summary

Biological invasions
- enemy-release and parasite mediated competition
- squirrels as invaders
- grey vs. red

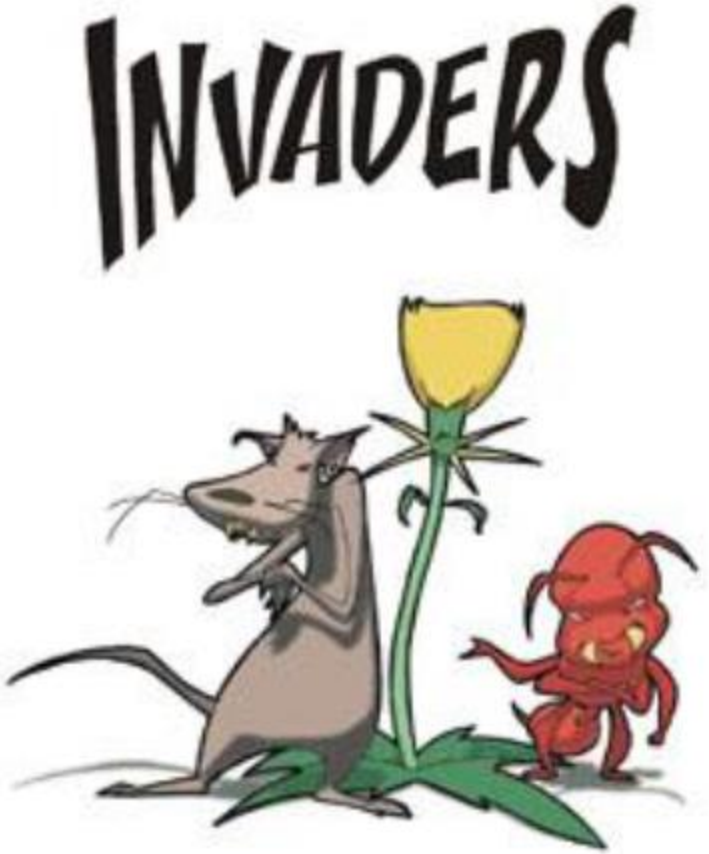
Squirrels & Parasites
- red, grey and Pallas's squirrels
- Macroparasites (overview and life cycles)

Methods
- CMR
- Tape-test, faecal egg count, floatation
- Post-mortem
- Arena test

Case studies

Extra: physiological stress

Credits Ralph Underhill



A species that has been introduced to an environment where it is non-native, or alien, and whose introduction causes environmental or economic damage or harm to human health

Biological invasions

INTRODUCED SPECIES → High impact → **INVASIVE SPECIES**

Decline in **population size**

Habitat degradation or replacement

Inhibits growth of other species

Predation

Interference with **reproduction**

Loss of **genetic diversity**

Affect plan/animal **health**

PARASITES TRANSMISSION to NATIVE SPECIES

1. Parasite release

ENEMY - RELEASE

Invaders lose part of their parasites during invasion

Invaders benefit: fitness, demographic growth, competitive abilities

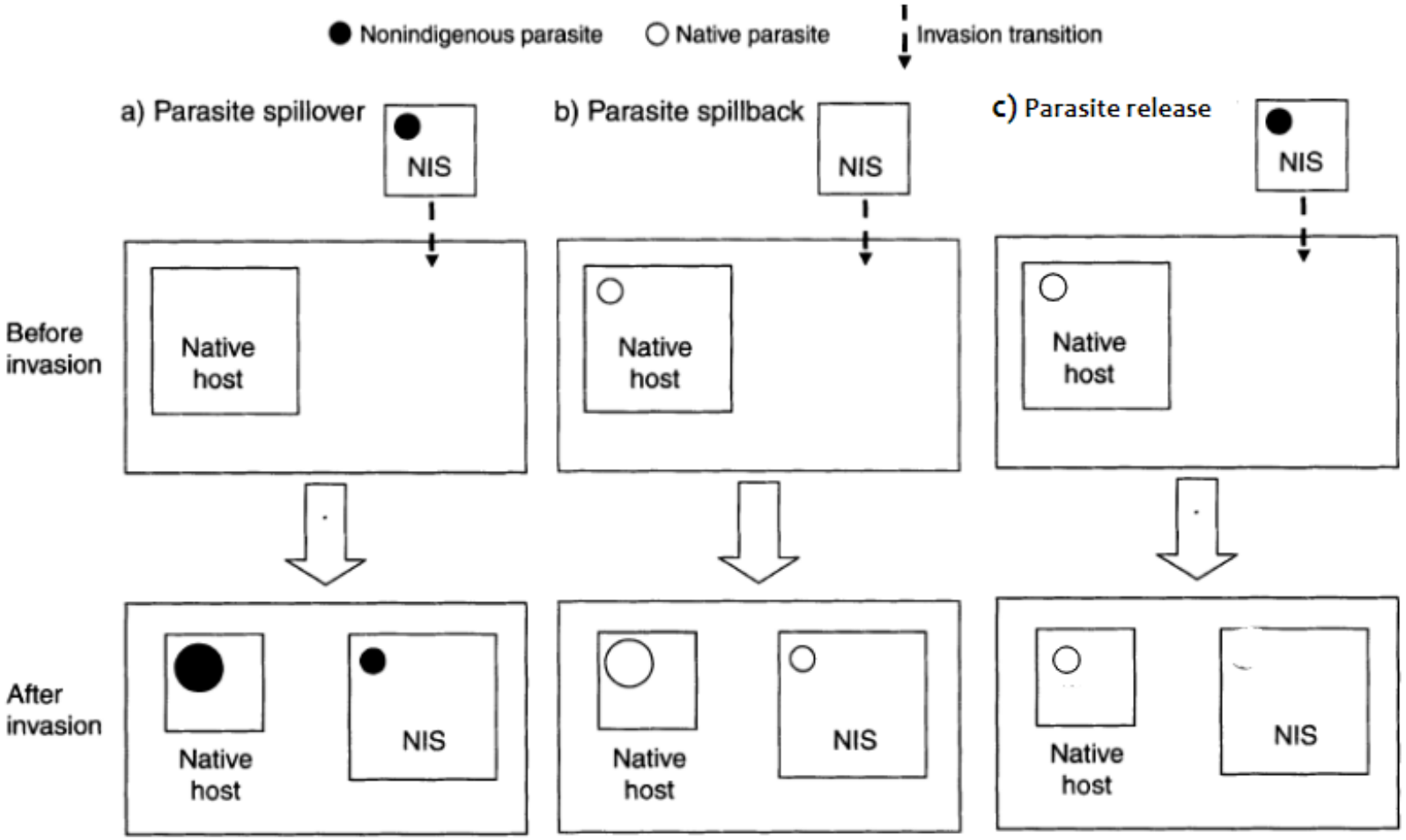
Invaders higher performance in the introduction than native range

Tompkins et al. 2002; Prenter et al. 2004; Dunn et al. 2012

Parasite mediated competition

2. Introduction alien parasites	SPILLOVER	Transmission of parasites carried by IAS host to the native host
3. Acquisition of local parasites	SPILL-BACK	Invasive species is a suitable host for native parasites (retransmission to the native species)

Tompkins et al. 2002; Prenter et al. 2004; Dunn et al. 2012



Tompkins et al. 2002; Prenter et al. 2004; Dunn et al. 2012

Tree squirrels as invaders

Dispersal: great distances, cross waterways, agricultural/urban areas

Nests: natural cavities in the trees or in the tree canopy (leaves, branches)

Diurnal with one or two activity peaks depending on daylength

Food: high variety (seeds, nuts, flowers, mushrooms, insects)

Food storage: small round holes on the ground or natural cavities in the trees

Plasticity in human-impacted landscapes

High reproductive potential (1-2 litters/year)

Habitat

Mountain conifer forests

Deciduous forests

Urban parks

Grey vs. Red

Niche overlap ~70%

COMPETITION FOR FOOD

COMPETITION FOR SPACE

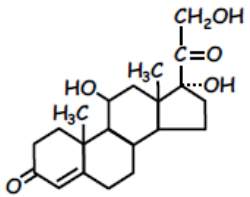
COMPETITION MEDIATED BY PARASITES

- decrease in reproductive rate (- F oestrus)
- juvenile recruitment (- juv survival)
- decrease male residency

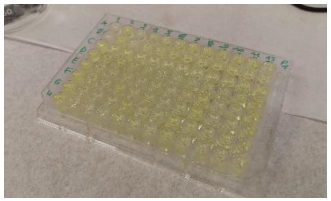
RED SQUIRRELS LOCAL EXTINCTION (4-10 years)

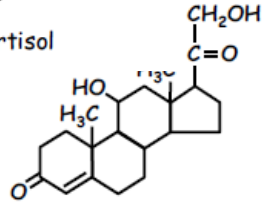
Wauters et al. 2002, 2005; Gurnell et al. 2004

Grey vs. Red



Cortisol





Corticosterone

Grey squirrel presence induces increase in red squirrels stress hormones (FGMs)

Red squirrels co-occurring with the alien species had higher sociability than in red-only sites (personality)

Red squirrels behavioural syndrome (activity-exploration-social tendency) disrupted in red-grey sites (personality)

Santicchia et al. 2018; Wauters et al 2019; Santicchia et al. submitted

Alien squirrels in Italy

Sciurus carolinensis

Callosciurus erythraeus

Callosciurus finlaysonii

Tamias sibiricus

Eurasian red squirrel

Sciurus vulgaris

- Densities 0.5-1.5 ind/ha
- Territoriality: intrasex (females defend core area from other females) and intersex (males home range partly overlap with females home range)
- Solitary
- Habitat: lowland deciduous woods, mountain conifer forests, urban parks

Eastern grey squirrel

Sciurus carolinensis

Introduced from N. America

- Densities from <3 to >21 ind/ha
- Territoriality not evidenced (extensive home ranges overlap)
- Kin groups (females are organized in social units of related animals that defend a discrete area)
- Habitat: overlap with native species

Pallas's squirrel

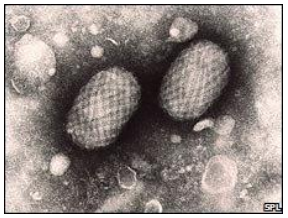
Callosciurus erythraeus

Introduced from Asia

- Maximum densities 5.2 ind/ha
- Territoriality not evidenced (extensive home ranges overlap)
- Habitat: overlap with native species (high tree density and canopy cover, vertical layers of canopy cover)

PARASITES

Microparasites
(viruses, bacteria, fungi, protozoa)



Macroparasites

Ectoparasites

- Fleas
- Ticks
- Lices
- Mites
- ...

Endoparasites

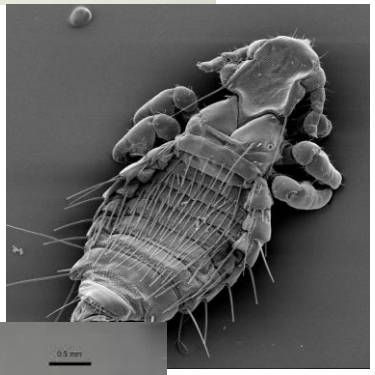
- **Nematodes**
- ...

Macroparasites of red squirrels

Endoparasites	
	Prevalence
Trypanoxyuris (R.) sciuri	**87%**
Strongyloides robustus	< 5%
Trichostrongylus sp.	< 5%
Capillariinae [gen. sp.]	< 5%
Hymnolepididae *[gen. sp.]*	< 5%

Romeo et al. 2013

	Ectoparasites	
		Prevalence
FLEAS	***Ceratophyllus (M.) sciurorum***	**27%**
	Tarsopsylla o. octodecimdentata	< 5%
	Dasypsyllus (D.) gallinulae	< 5%
LICES	*Neohaematopinus sciuri*	8%
	Enderleinellus nitzschi	< 5%
TICKS	***Ixodes (I.) ricinus***	**34% (France)**
	Ixodes (I.) acuminatus	**6,5% (Italy)**



Romeo et al. 2013

- *Trypanoxyuris* (*Rodentoxyuris*) *sciuri*

- Oxyurid nematode specific to the red squirrel
- Large intestine and caecum
- Direct life-cycle without free living stages
- Horizontal trasmission possible

Prevalence (± SE)	Mean abundance (worms/host)	Range (worms/infected host)
97% ± 3%	**460 ± 99**	**1 - 5227**

Romeo et al. 2013; Santicchia et al. 2015

Eggs

Grooming

Anus

Eggs ingestion

Stomach

Rectum

Small intestine

Large intestine

Larva type 1

Larva type 2

Pregnant female

Adult female

Adult male

Macroparasites of grey squirrels (Italy)

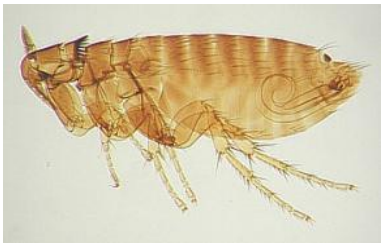
Grey squirrel dominant helminth

SPILLOVER
to native species

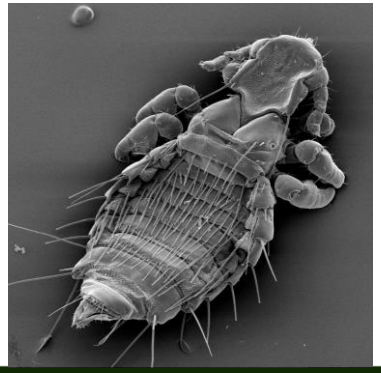
Endoparasites	
	Prevalence
Strongyloides robustus	**61%**
Trichostrongylus calcaratus	12%
Trichuris muris	5%
Aonchotheca annulosa	4%
Trypanoxyuris (R.) sciuri	1%
Trichostrongylus retortaeformis	1%
Strongylida [gen. sp.]	4%
Oxyurida [gen. sp.]	1%

Romeo et al. 2014

	Ectoparasites	
		Prevalence
FLEAS	***Ceratophyllus (M.) sciurorum***	**21%**
	Ctenocephalides felis felis	1%
LICES	*Neohaematopinus sciuri*	14%
TICKS	*Ixodes acuminatus*	1%



SPILL-BACK ???



Macroparasites: grey squirrel dominant helminth

- *Strongyloides robustus*

Strongyloides spp. adult and egg

- Grey squirrels: 61/95 infected by *S. robustus* (prevalence 60 ± 4%)
- Abundance: 0 to 86 helminths per host (mean ± SE = 6.68 ± 1.36)
- Mean Intensity of *S. robustus* = 10.4 ± 2.0 helminths per host

Romeo et al. 2014

Hatch in the environment where larvae develop (L1, L2, L3)

S. robustus eggs are shed with host faeces

Larvae infect a new host by penetrating its skin

Adult phase in host gastro-intestine

Romeo et al. 2014, 2015

Parasite release: grey squirrels in Italy

- Endoparasite fauna dominated by a single species (*Strongyloides robustus*)
- Low parasite richness
  - few founding individuals
  - loss during establishment
    - low host densities
    - environmental conditions
  - founders from pet shops (antiparasitic treatments)

Competitive advantage for the invader over the native

Macroparasites of Pallas's squirrels (Italy)

Red squirrel dominant helminth

~~SPILL-BACK~~

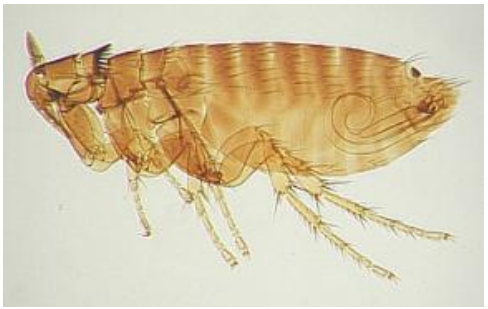
~~SPILLOVER~~

Endoparasites	
	Prevalence
Trypanoxyuris (R.) sciuri	**5%**
Trichuris muris	4%
Strongyloides callosciureus	**1%**
Strongyloides sp.	1%
Capillariinae	1%
Spiruridae	1%

Mazzamuto et al 2016

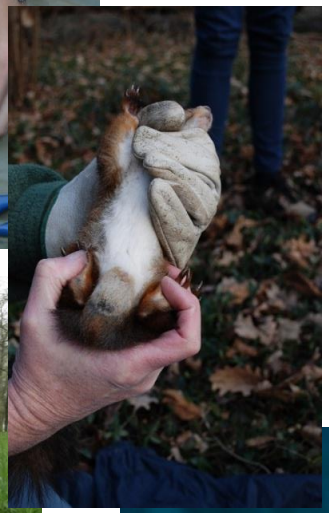
SPILL-BACK ???

	Ectoparasites	
		Prevalence
FLEAS	*Ceratophyllus (M.) sciurorum*	50%
TICKS	*Ixodes ricinus*	47%
MITES	*Trombiculidae*	7%



Mazzamuto et al 2016

Methods: fieldwork



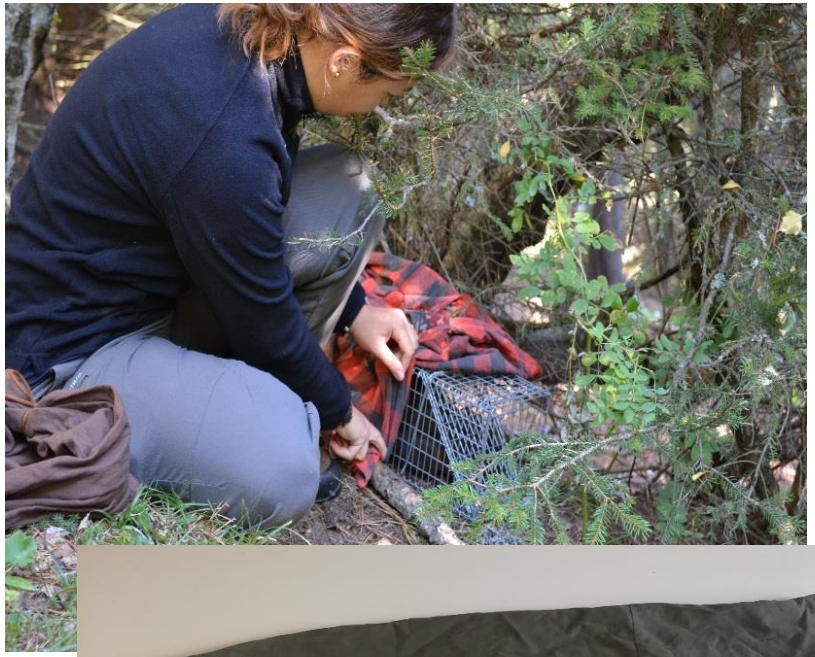
Capture-mark-recapture:

- Ear tag
- Body mass
- Hind foot length
- Sex and reproductive condition
- Collection of samples
- Arena test

Capture-mark-recapture: CMR

-first step: cover the trap with a cloth to reduce stress

Capture-mark-recapture: CMR



-zipper-tube handling bag

Capture-mark-recapture: CMR

-reduce contact with the operator

-body mass

Capture-mark-recapture: CMR

-individual mark with ear tag

Capture-mark-recapture: CMR

-radio-collar positioning

Capture-mark-recapture: CMR

- Hind foot length

Capture-mark-recapture: CMR

- Sex and reproductive condition

Faecal samples

Tape-test

Arena test

OPEN FIELD TEST (OFT)

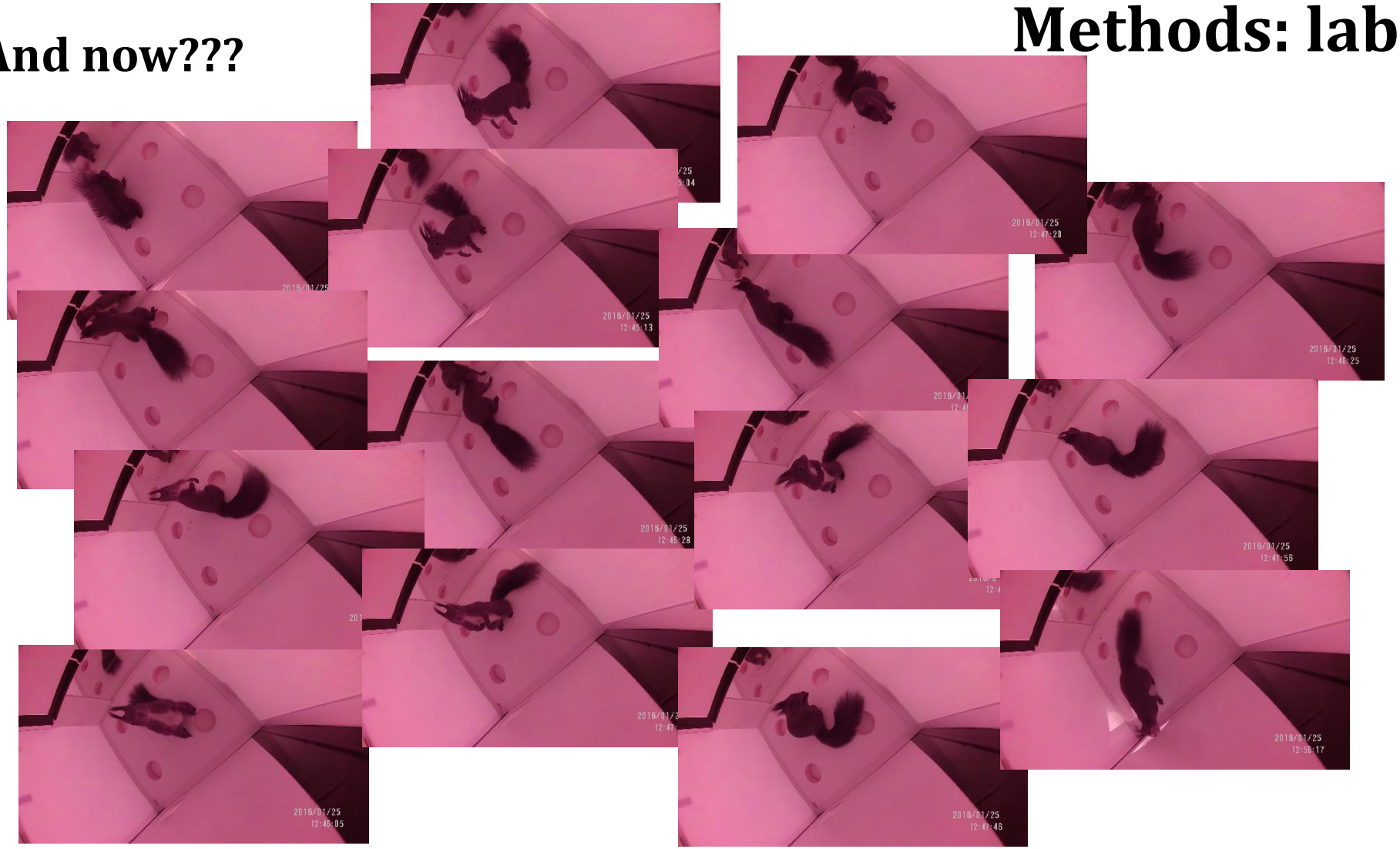
Activity and exploration in a novel environment

MIRROR IMAGE STIMULATION TEST (MIS)

Reaction to conspecifics

Methods: lab

And now???



Ethogram: previous studies

Behaviour	OF1	OF2	OF3	Behaviour	MIS1	MIS2
Still	**−0.49**	−0.11	−0.07	Attack rate**	**0.42**	−0.13
Walk	**0.48**	0.10	−0.13	Crouch rate††	**0.42**	0.03
Jump rate	**0.38**	**0.36**	−0.13	Front‡‡	**0.42**	−0.01
Sniff*	**0.34**	**−0.34**	−0.11	Attack latency	**−0.40**	**0.41**
Hole rate†	**0.29**	**−0.34**	−0.26	Approach latency	**−0.39**	0.00
Rear	**0.29**	−0.24	−0.05	Back‡‡	**−0.30**	**−0.35**
Hang‡	0.18	**0.55**	0.28	Grunt§§	0.22	−0.10
Chew§	0.08	**−0.30**	**0.56**	Stretch¶¶	0.15	**0.82**
Scan	−0.03	0.26	**−0.49**			
Groom	−0.0					
No. pellets¶	0.2					

Open Field Trial		Mirror Image Stimulation Trial	
Behaviour	**Behavior description**	**Behaviour**	**Behavior description**
Chew/dig	Chew or scratch floors or walls	Chew/dig	Chew or scratch floors or walls
Climb/hang	Climbing or hanging on walls	Climb/hang	Climbing or hanging on walls
Grooming	Grooming activity	Crouch	Attack imminent - tail over back with hairs erect
Head dip	Dip head into blind holes	Grooming	Grooming activity
Immobile	No movement	Locomotion	Jump, walk
Locomotion	Jump, walk	Non-aggressive	non aggressive contact with mirror
Rear	Rising up on hind legs	Number attacks	Count of attacks on mirror
Scan	Head moving, rest of body immobile	Rear	Rising up on hind legs
Sniff	Sniff floor or walls	Scan	Head moving, rest of body is immobile
		Slow approach/stretch	Slow approach towards mirror, back legs stretched out behind
		Sniff	Sniff floor or walls
		Still in back	Immobile in back half of arena furthest from mirror
		Still in front	Immobile in front half of arena closest to mirror

Expert opinion

Ethogram development: recurrent behaviour identification

Open Field Test (OFT)		**Mirror Image Stimulation Test (MIS)**	
Behavior	**Behavior description**	**Behavior**	**Behavior description**
Scratch	Scratch or chew floors or walls	Scratch	Scratch or chew floors or walls
Hang	Hang on walls	Hang	Hang on walls
Groom	Grooming activity	Groom	Grooming activity
Locomotion	Jump, walk	Locomotion	Jump, walk
Rise	Rise up on hind legs	Rise	Rise up on hind legs
Scan	Head moving	Scan	Head moving
Sniff	Sniff the corner of arena	Sniff	Sniff the corner of arena
Head dip	Put head in holes in the floor	Head dip	Put head in holes in the floor
Immobile	No movement	Back	Immobile in back half of arena furthest from mirror
		Front	Immobile in front half of arena closest to mirror
		Slow	Slow approach towards mirror, with hind legs stretched out behind
		No-aggressive	Non aggressive contact with the mirror
		Attack	Strike the mirror with front legs or head
		Watch	Immobile, watching directly to mirror

Video coding

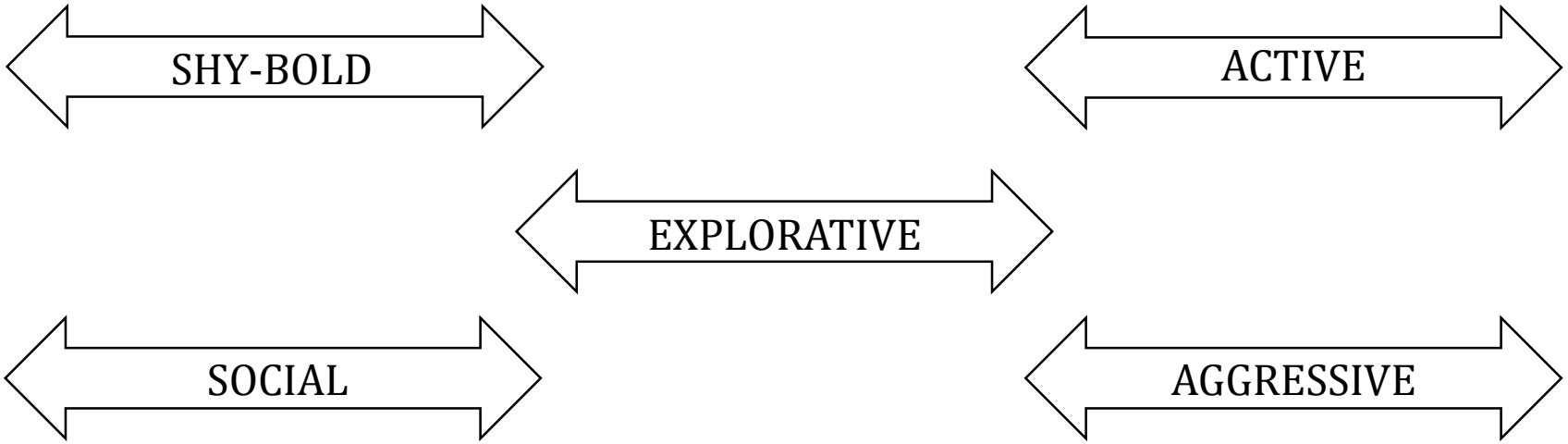
A lot of different available software!

CowLog

Software	Company/Developer	Category	Cost
BORIS	University of Turin	Event Coding	Free
CowLog	University of Helsinki	Event Coding	Free
Ethowatcher	Federal University of Santa Catarina	Event Coding	Free
LongoMatch	LongoMatch	Event Coding	Free
Observer XT 12	Noldus	Event Coding	~$2,500 + add-ons
Dartfish Connect 7	Dartfish	Measurement	Base Package: $1,000
Kinovea	Kinovea	Measurement	Free
Tracker	Cabrillo College (Douglas Brown)	Measurement	Free

Personality: Animal personality refers to among-individual differences in behaviour that persist through time and in different contexts

Personality trait: A specific aspect of a behavioural repertoire that can be quantified and that shows among-individual variation and within-individual consistency.



Réale et al. 2007; Carter et al. 2013.

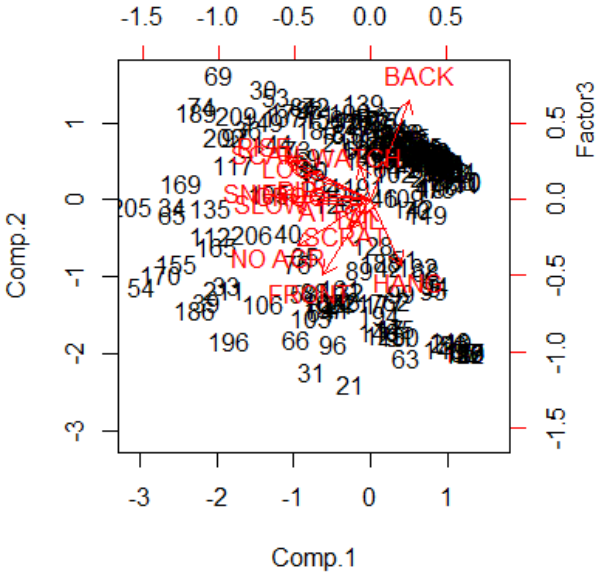
Behaviours ⟶ Personality traits

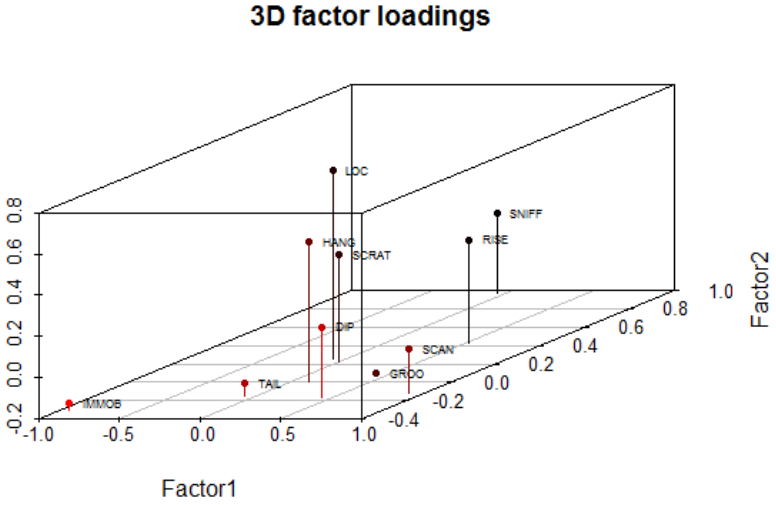
CowLog output file – POST-PRODUCTION with R

Reduction of behaviours in few personality-linked variables

Variable reduction with Multivariate Analysis:

- Principal Component Analysis (PCA)
- Factor Analysis (FA)
- Cluster Analysis
- ….





Expert-based behaviour groups

Reduction of behaviours in few personality-linked variables

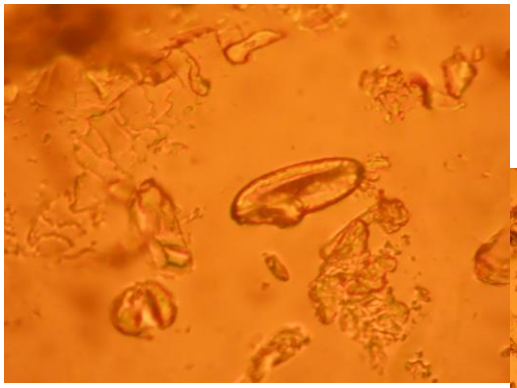
Expert-based group	Behaviors
OFT	
ACTIVITY	Locomotion, Rise, Scan
EXPLORATION	Sniff, Head dip, Scratch
SHYNESS	Immobile, Hang
MIS	
SOCIABILITY	Front, Slow, No-aggressive
AVOIDANCE	Back, Hang
ALERT	Watch
ACT-EXPL (OTHER)	Rise, Locomotion, Sniff, Head dip, Scratch, Scan

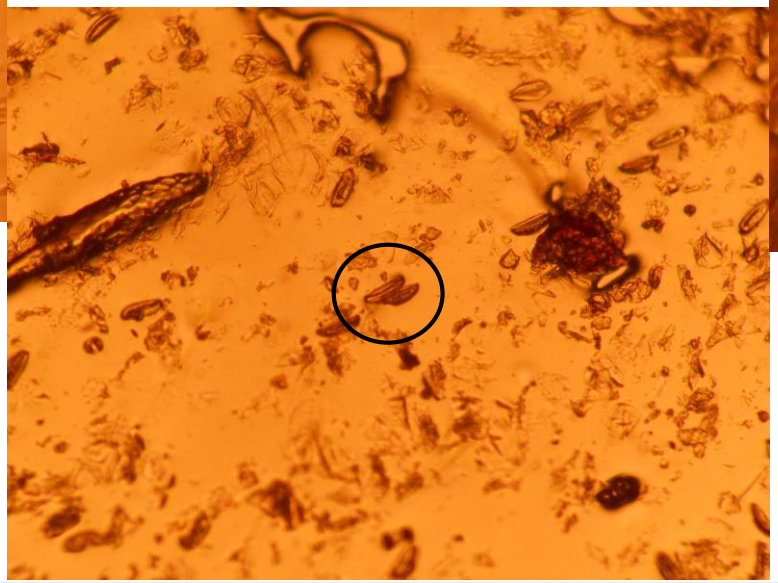
Mazzamuto et al 2018; Wauters et al 2019; Santicchia et al 2020.

Methods: lab

Tape-test →

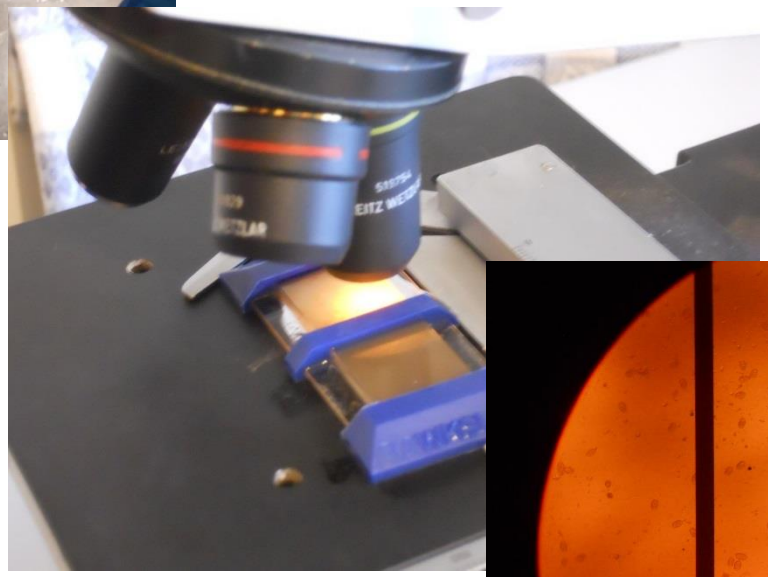
presence-absence
Trypanoxyuris (Rodentoxyuris) sciuri

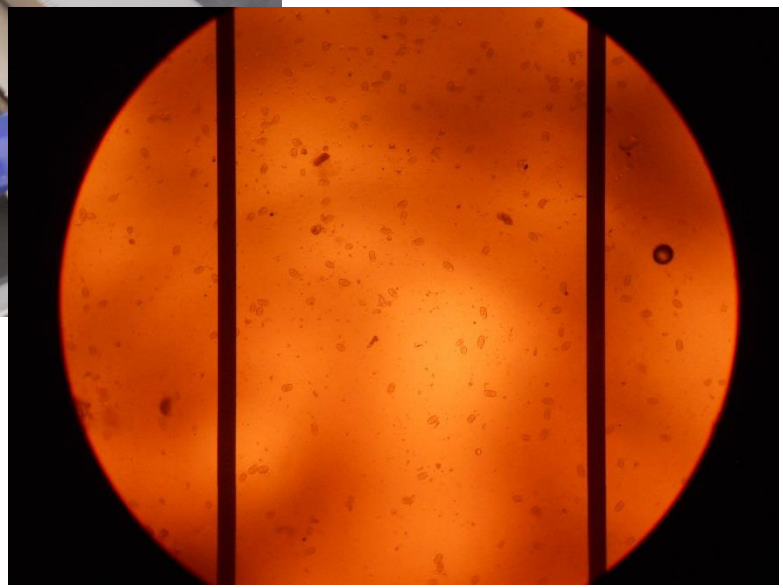




- QUALITATIVE: floatation
(presence/absence of helminth eggs)

Romeo et al. 2013; 2014a, b; 2015

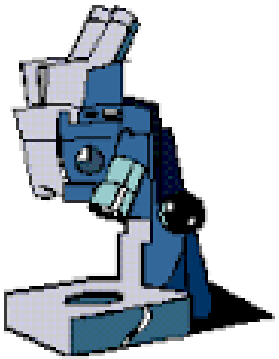




- QUANTITATIVE:
faecal egg count (FEC)
(helminth eggs/g
faeces)

Romeo et al. 2013; 2014a, b; 2015

Parasite load: *Post mortem* examination



Remove the GI and divide stomach, small intestine, large intestine and rectum

Open each tract and flush through a sieve

Examine the content using a stereo-microscope

Count helminths

Romeo et al. 2013, 2014

Case studies: habitat quality & parasites

Effects of habitat quality on parasite abundance: do forest fragmentation and food availability affect helminth infection in the Eurasian red squirrel?

Hyp:

Abundance of *T. sciuri* x habitat-type, fragmentation, food availability

Methods:

Post-mortem examination of road-killed red squirrels

Food availability: cone count data of Norway spruce (*Picea abies*) from mountain habitat

Santicchia et al. 2015 *J Zool* doi:10.1111/jzo.12215

Conifer forests

Norway spruce (*Picea abies*) – Abete rosso
Arolla pine (*Pinus cembra*) – Pino cembro
Larch (*Larix decidua*) - Larice
Mountain pine (*Pinus mugo*) – Pino mugo

Pulsed resource system

Squirrel population dynamic

Seeds pre-dispersal predation

Seeds (survival-germination)

Cone count

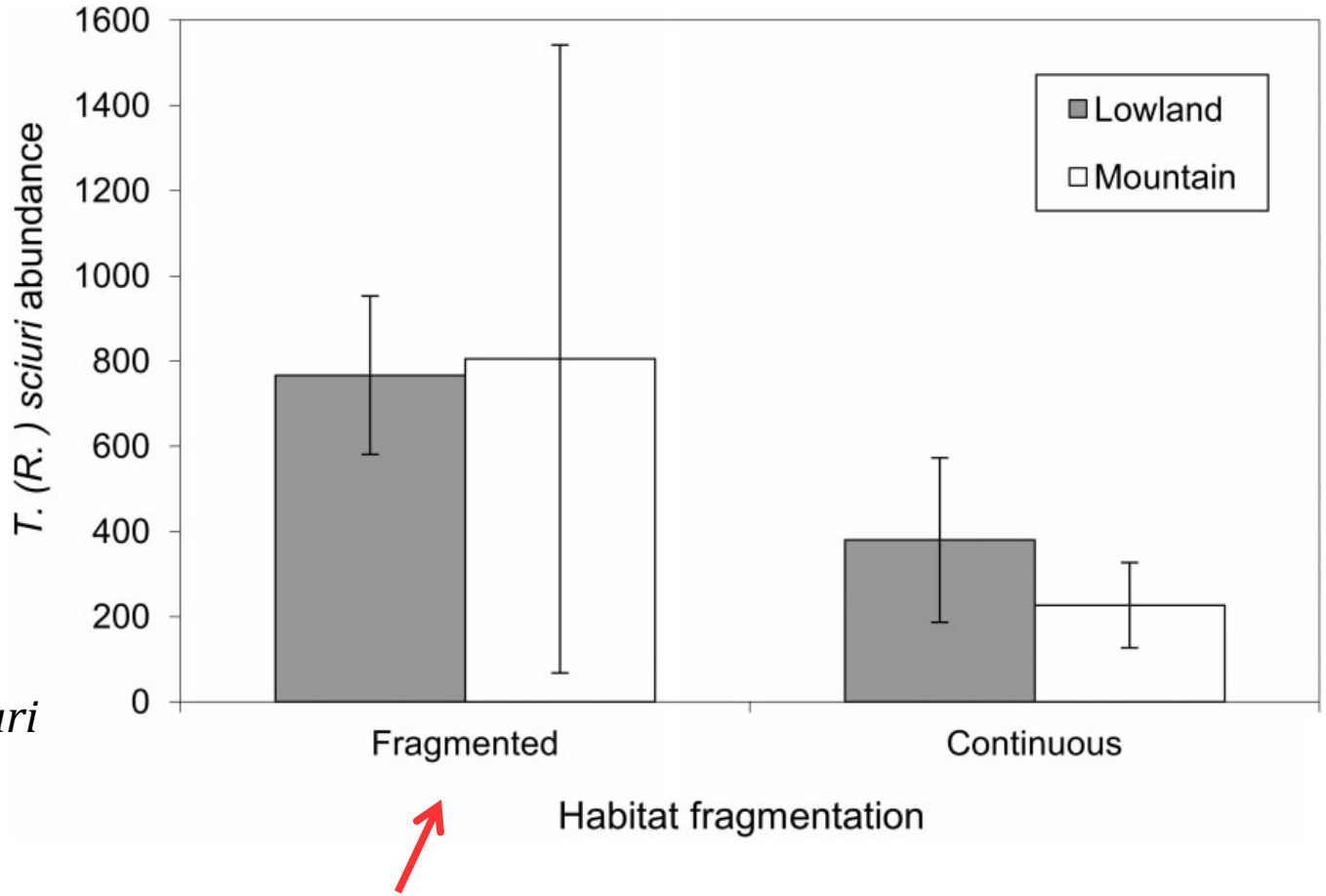
Larix decidua

Pinus mugo

Pinus cembra

Picea abies

Mean abundance (number of worms per host) of *T. sciuri*



Abundance of *T. sciuri* increased in fragmented woods

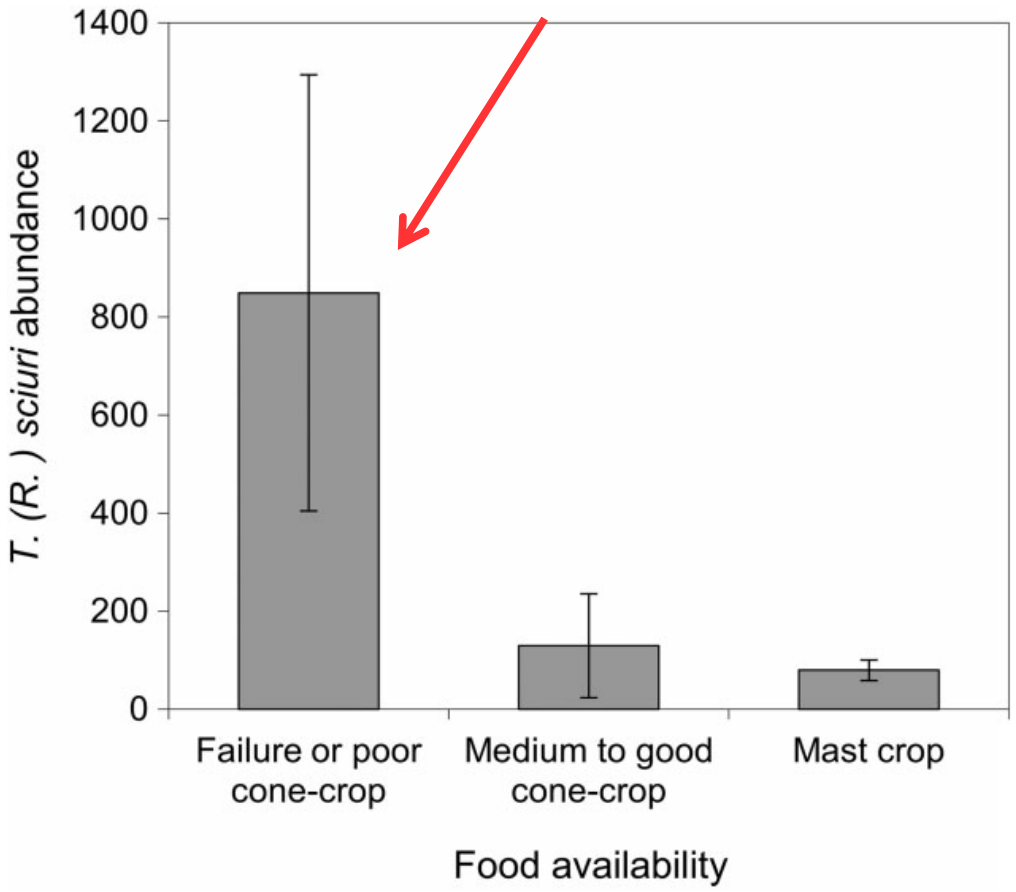
Santicchia et al. 2015 *J Zool* doi:10.1111/jzo.12215

Mean abundance (number of worms per host) of *T. sciuri* in mountain habitat

Squirrels were more heavily infected after a poor Norway spruce seed crop than in years with medium or high seed production

↓

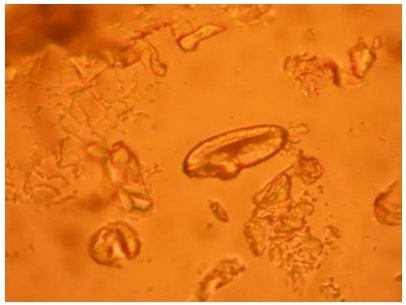
less capable of reducing parasite load when food availability is low



Santicchia et al. 2015 *J Zool* doi:10.1111/jzo.12215

Case studies: *S. robustus* spillover

Biodiversity threats from outside to inside: effects of alien grey squirrel (*Sciurus carolinensis*) on helminth community of native red squirrel (*Sciurus vulgaris*)



Trypanoxyuris sciuri egg

Strongyloides robustus egg

Hyp:

? Spillover of *S. robustus* occurs

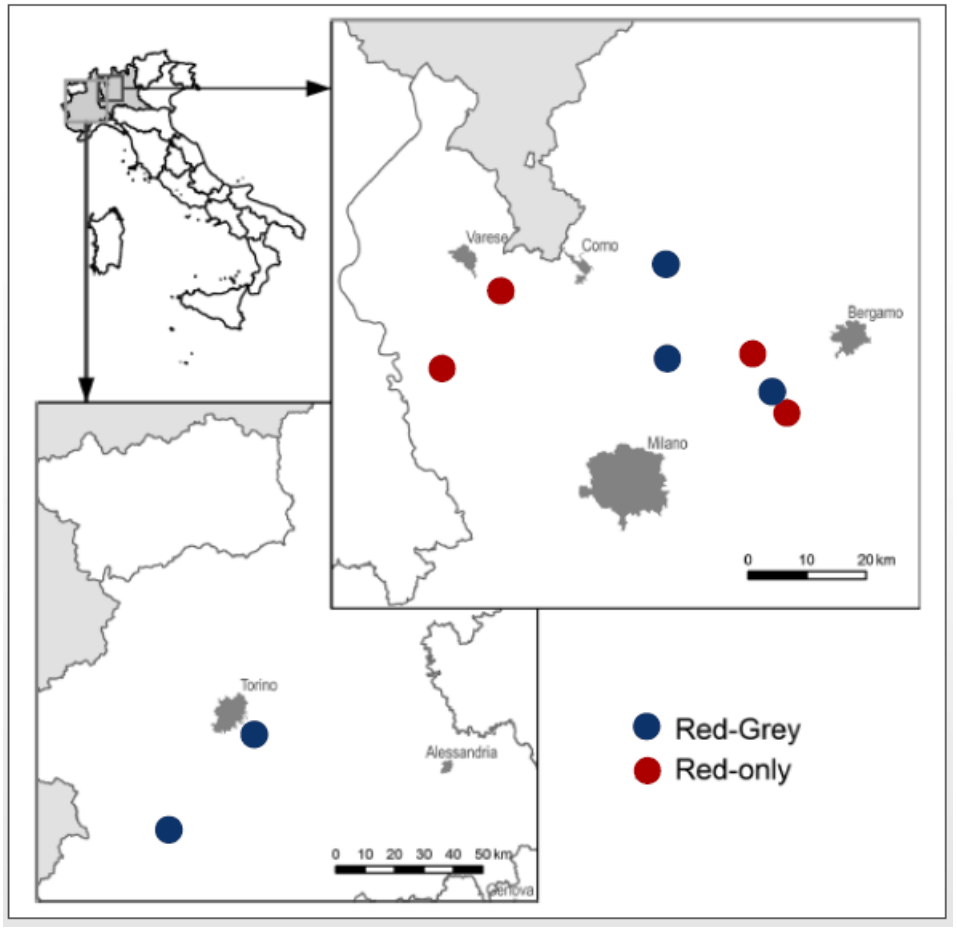
? Prevalence of *T. sciuri* in red squirrels is affected by grey squirrel presence

Methods:

Floatation (presence-absence) *Strongyloides robustus*

Tape-test (presence-absence) *Trypanoxyuris sciuri*

Romeo et al. 2015 *Parasitol Res* doi:10.1007/s00436-015-4466-3

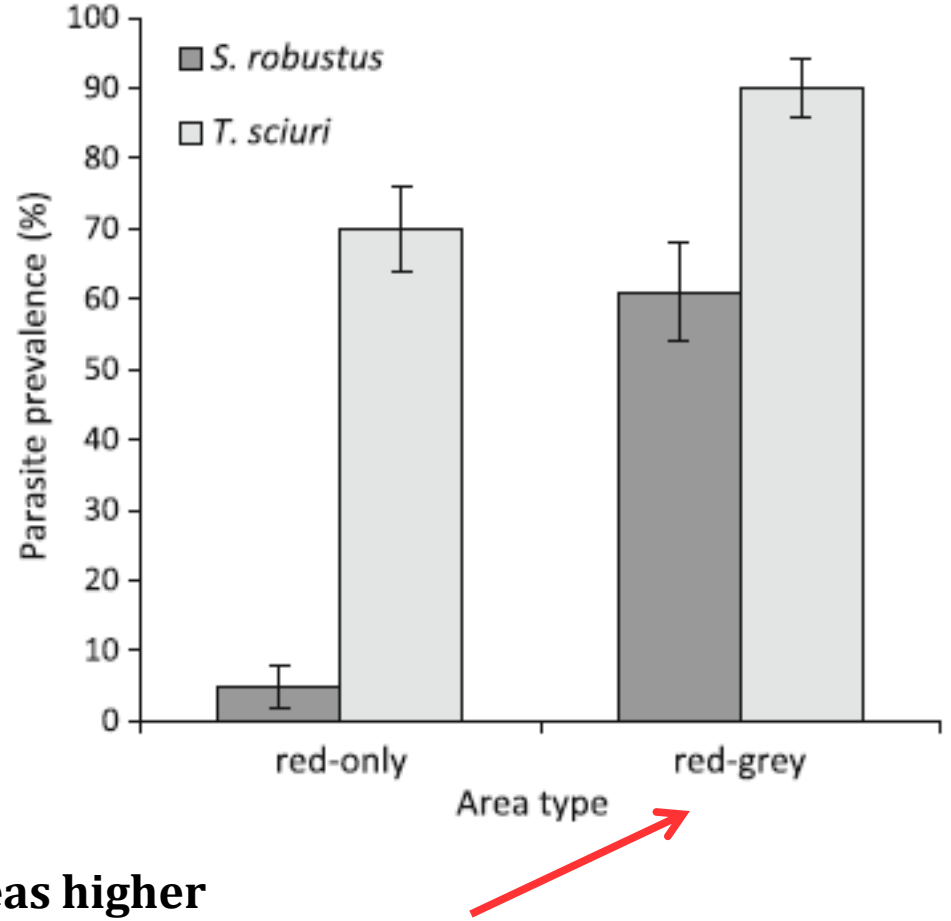


4 **red-only** sites
5 **red-grey** sites

Extensive **live-trapping** (2011-2013)

Romeo et al. 2015 *Parasitol Res* doi:10.1007/s00436-015-4466-3

In areas co-inhabited by grey squirrels, **red squirrels have a higher probability of being infected by *S. robustus* and *T. sciuri***



Spillover!

In red-grey areas higher infection by *T. sciuri*

Romeo et al. 2015 *Parasitol Res* doi:10.1007/s00436-015-4466-3

Case studies: personality & parasites

The price of being bold? Relationship between personality and endoparasitic infection in a tree squirrel

Hyp:

? Squirrels personality influence infection dynamics

Methods:

Boldness-exploration determined through indirect indices of personality

Post-mortem examination of carcasses *Strongyloides robustus*

Santicchia et al. 2019 *Mamm Biol* doi:10.1016/j.mambio.2019.04.007

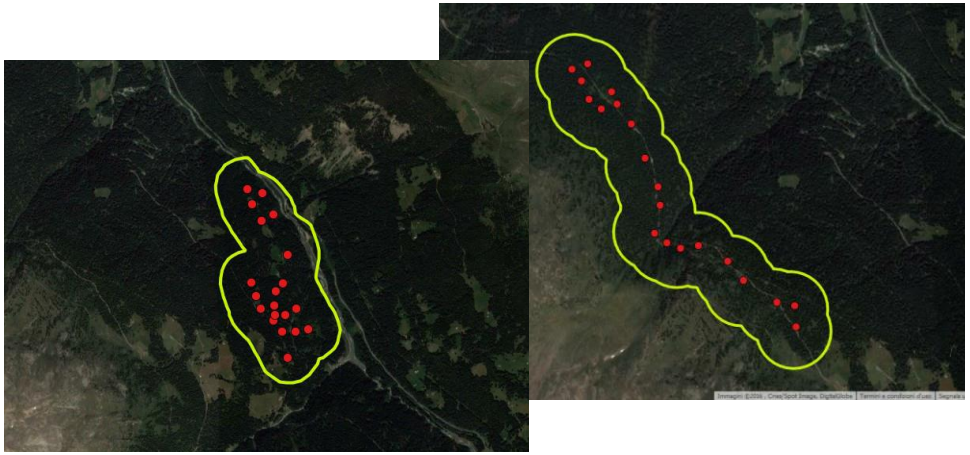
Parasites ⟷ host behaviour

Variation in behaviour among individuals in the host population

- Trasmission by contact rates among individuals
- Chances of encountering parasites infective stages in the environment

Ezenwa et al. 2016

Indirect indices (Capture-mark-recapture data)



TRAPPABILITY

Total number of captures/ length capture period

Propensity to take risks

BOLDNESS

TRAP-DIVERSITY

Number of different traps/ n. available traps

Propensity to explore

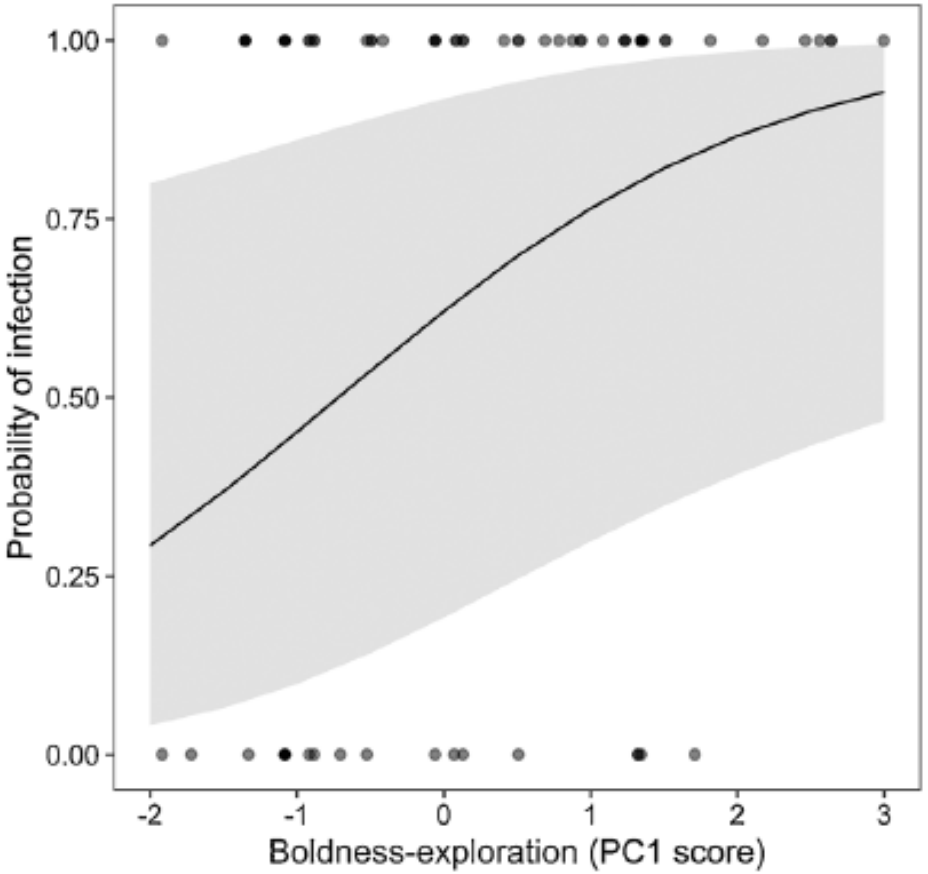
EXPLORATION

PC1 score
BOLDNESS-EXPLORATION

Santicchia et al. 2019 *Mamm Biol* doi:10.1016/j.mambio.2019.04.007

Infection status (0/1) affected only by **personality**

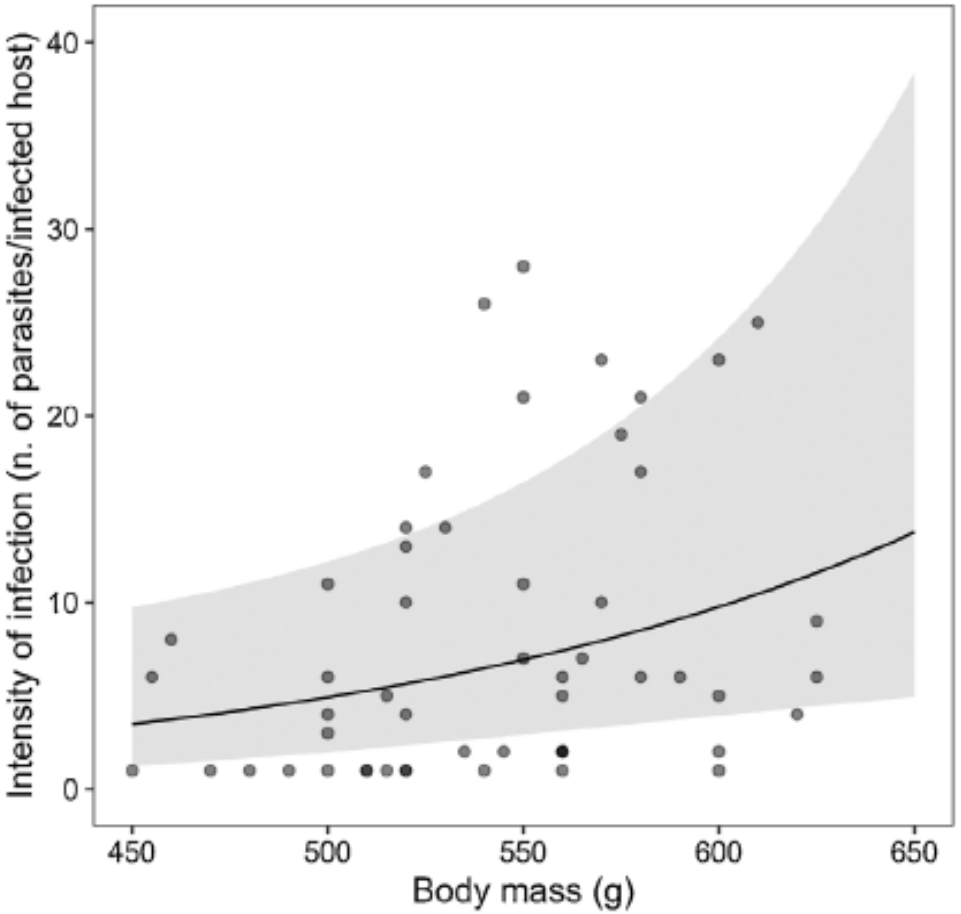
Bolder, more explorative grey squirrels are more likely to **acquire *S. robustus***



Santicchia et al. 2019 *Mamm Biol* doi:10.1016/j.mambio.2019.04.007

***S. robustus* intensity** affected only by **body mass**

Once infected, intensity of infection is determined by other mechanisms related to body mass

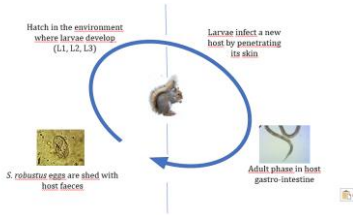


Santicchia et al. 2019 *Mamm Biol* doi:10.1016/j.mambio.2019.04.007

Implications in **biological invasions**
as **bolder, more explorative** individuals are more likely to be
the **first to invade** new areas and:

carry along and spread
alien parasites

acquire local parasites and
alter their circulation



+ chances for **SPILLOVER** or
SPILL-BACK to native species

Santicchia et al. 2019 *Mamm Biol* doi:10.1016/j.mambio.2019.04.007

Case studies: personality & parasites

Spillover of an alien parasite reduces expression of costly behaviour in native host species

Hyp:

? *S. robustus* spillover affects red squirrel's behaviour

Methods:

Capture-mark-recapture (3 red-only, 3 red-grey sites)

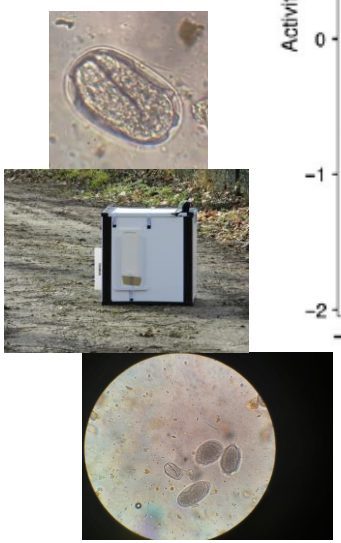
Faecal egg count (*Strongyloides robustus*)

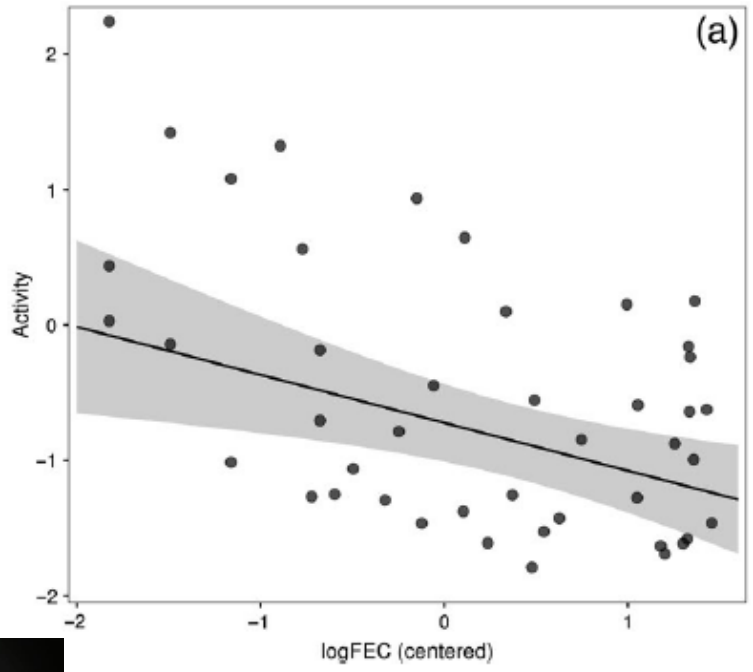
Tape-test (presence-absence)
Trypanoxyuris sciuri

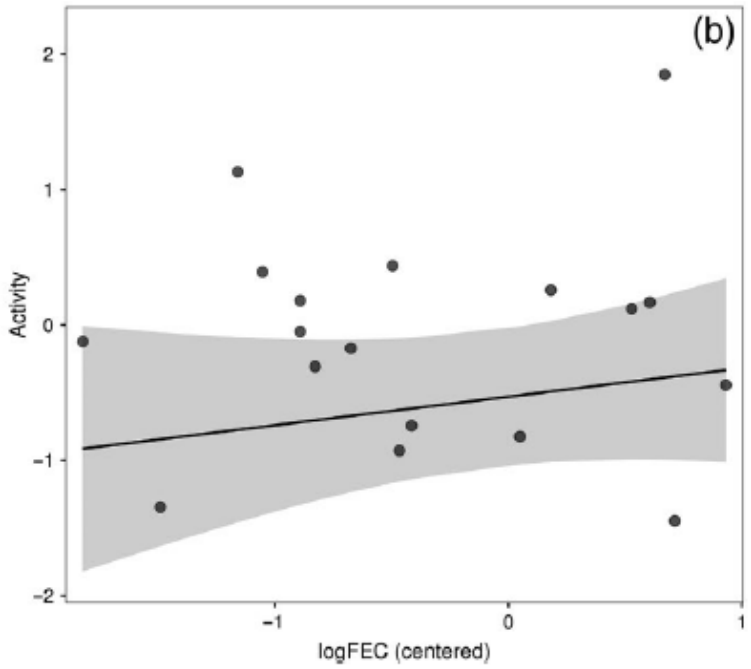
Arena test (personality traits)

Santicchia et al. 2020 *J Anim Ecol* doi:10.1111/1365-2656.13219

S. robustus egg count negatively associated with activity → Only in squirrels affected also by *T. sciuri*





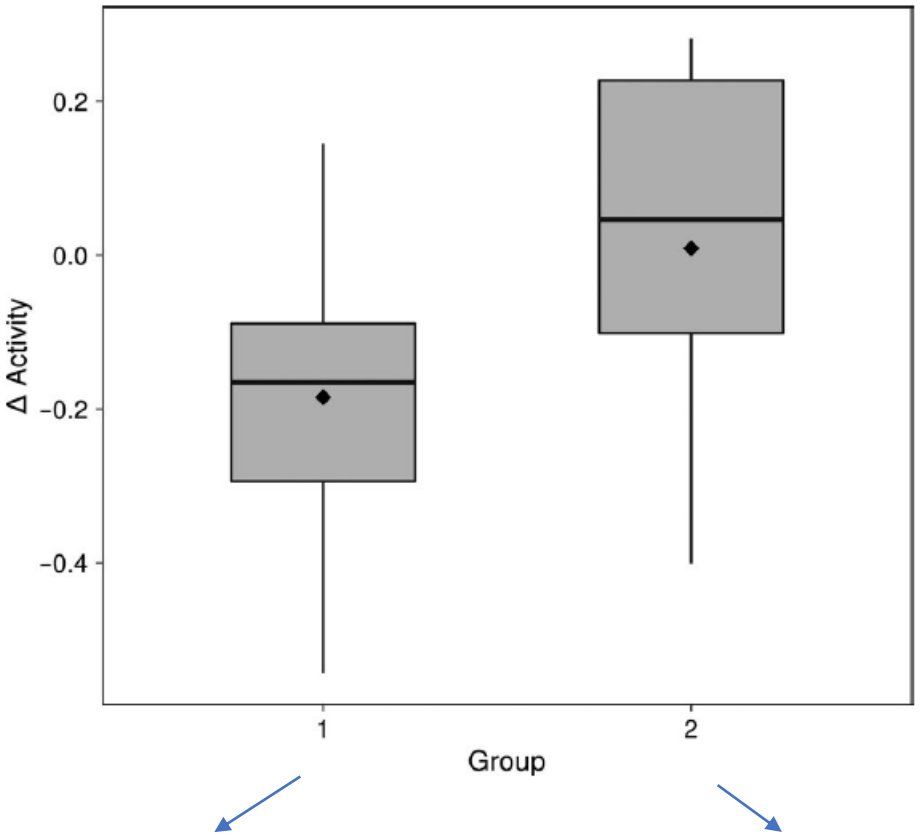


S. robustus + *T. sciuri*

only *S. robustus*

Santicchia et al. 2020 *J Anim Ecol* doi:10.1111/1365-2656.13219

decrease in **activity** levels following *S. robustus* infection



Not infected - **infected**

Not infected – **not infected**

Santicchia et al. 2020 *J Anim Ecol* doi:10.1111/1365-2656.13219

And so on….

Physiological stress

Immunity

Hologenomics
(http://www.earthhologenome.org/squirrel_hologenomics.html)

Urban ecology
(https://www.facebook.com/selvatiCitta/)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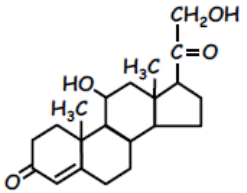
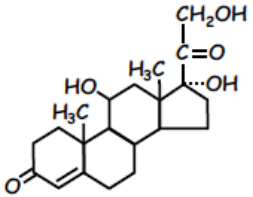
Glucocorticoid hormones

Involved in:
-body growth
-reproduction
-digestion

and
-expression of behaviour
-response to infection and disease



Corticosterone

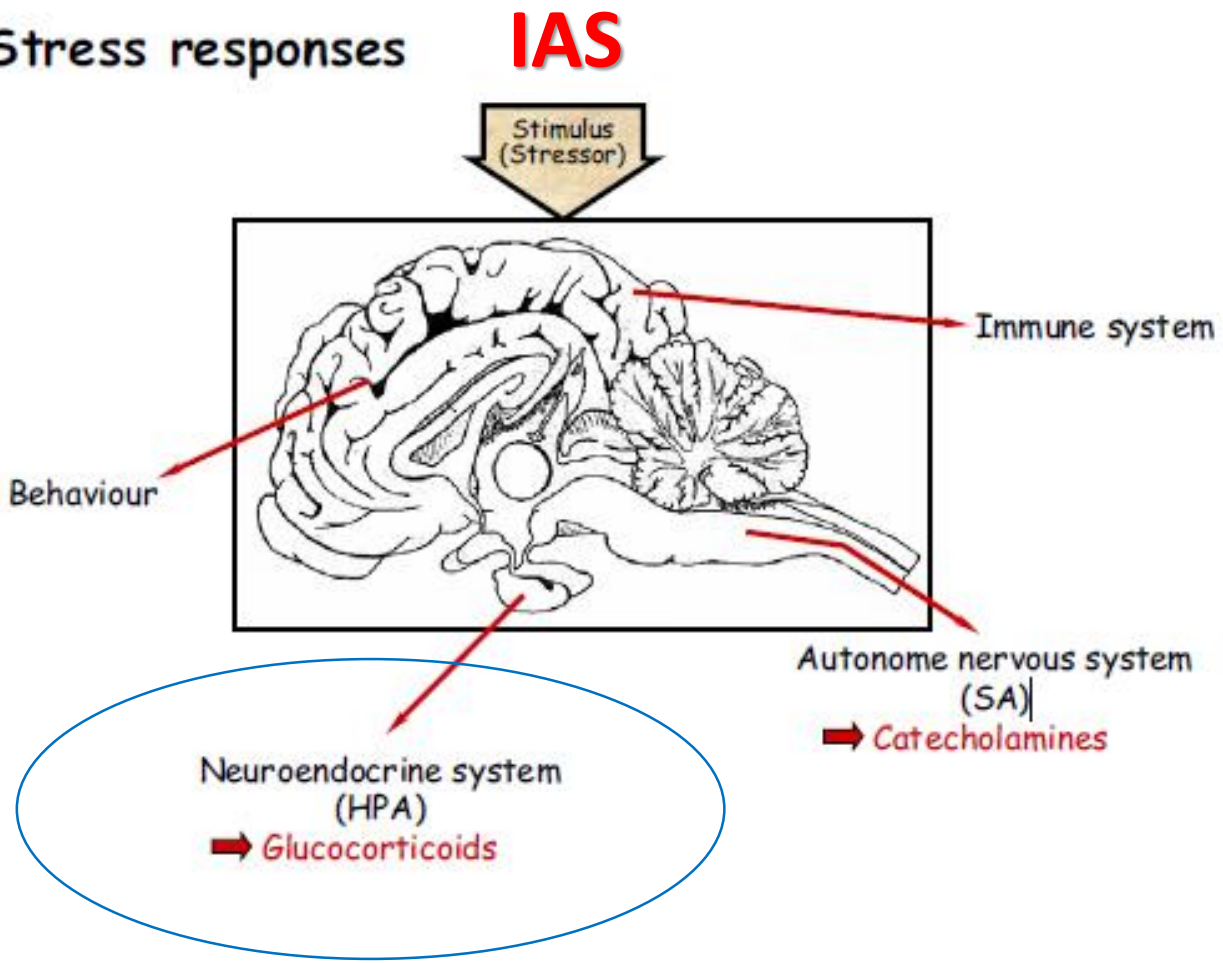


Cortisol

Chronically stressed individuals tend to experience a larger cumulative exposure to glucocorticoids

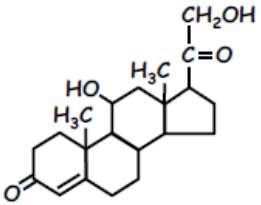
- urine
- hair
- blood
- **faeces**

Romero 2004.

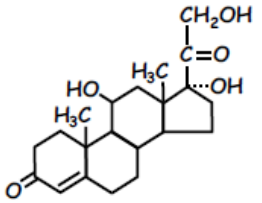

Stress responses
IAS
Stimulus
(Stressor)
Immune system
Behaviour
Autonome nervous system
(SA)
Catecholamines
Neuroendocrine system
(HPA)
Glucocorticoids

Palme 2010

High glucocorticoids



Corticosterone



Cortisol

Energy balance alteration

Inhibition of growth

Behaviour alteration

Effects on immunity

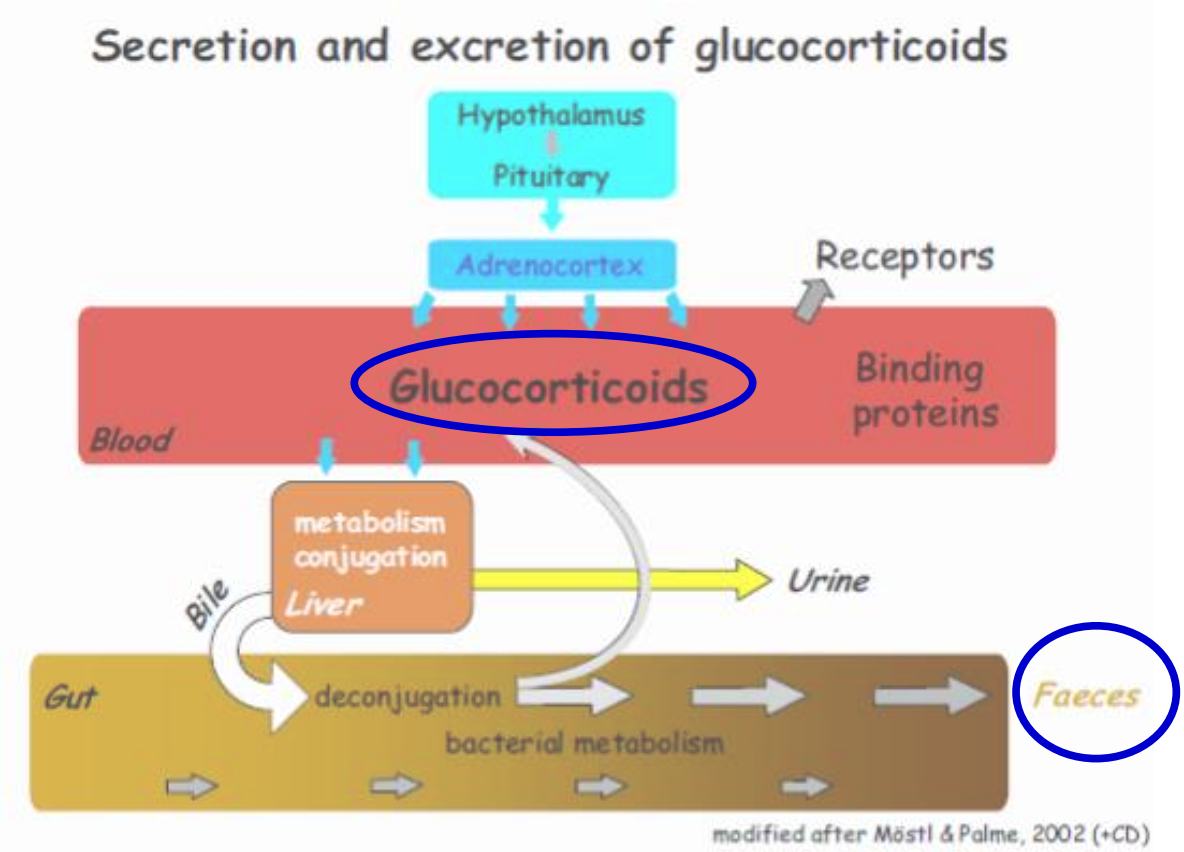
Effects on reproduction

Suppression of digestion

Increase in blood glucose levels

Inflammatory response

Decline survival/reproduction



Möstl, E., Palme, R. (2002): Hormones as indicators of stress. Dom. Anim. Endocrinol. **23**, 67-74.

FGMs (Faecal Glucocorticoid Metabolites)

- non-invasive technique

- useful for free-ranging animals

- technique already developed on many Sciurid species

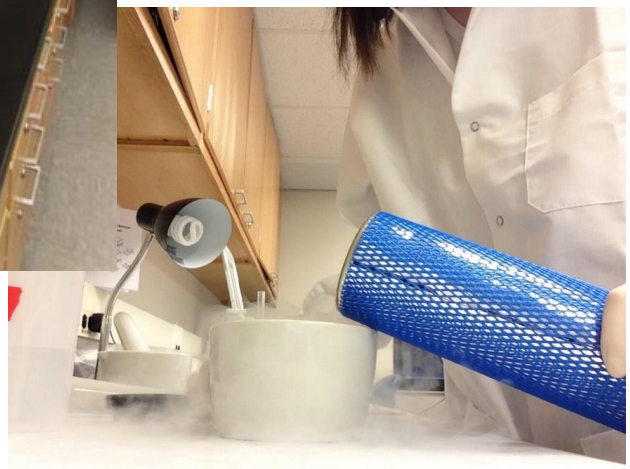
- validation for each species

Dantzer et al. 2010, 2014, 2016.

Hormones extraction

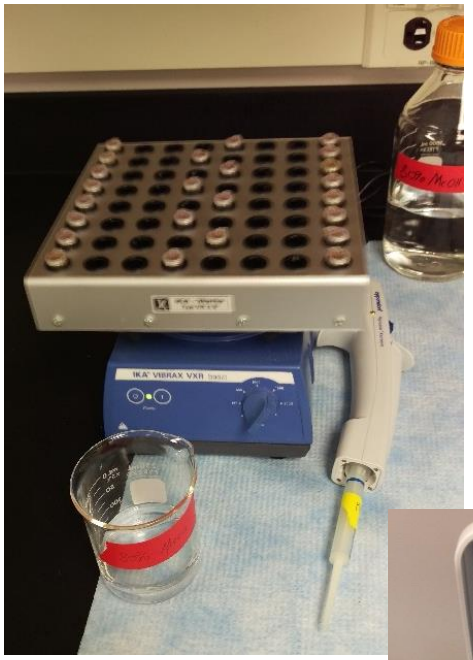
Lyophilization

- Wet faeces were dryied through lyophilizer (14-16 h)



Grinding and pulverization

- Lypophilized faecal samples were grinded in a mortar with liquid nitrogen

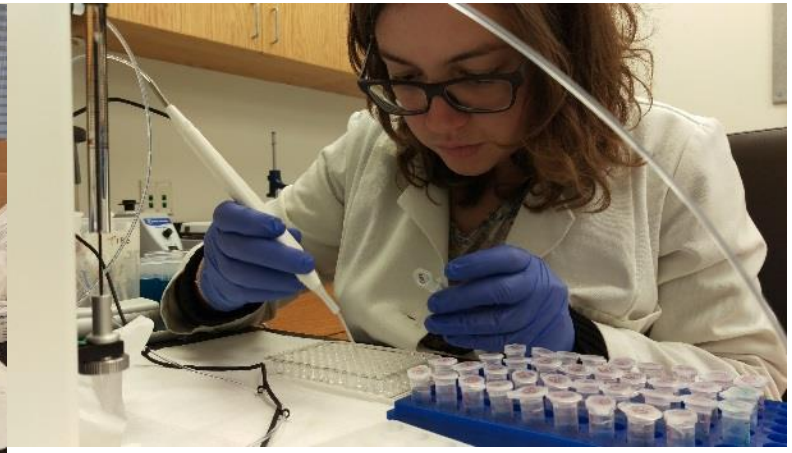


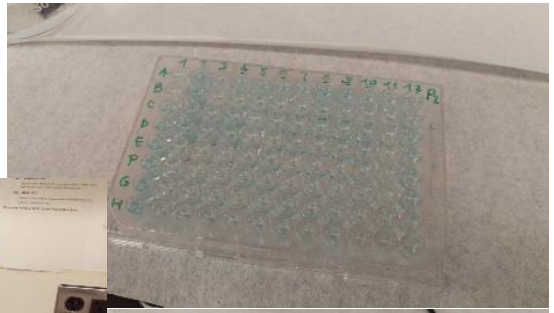
Mixing

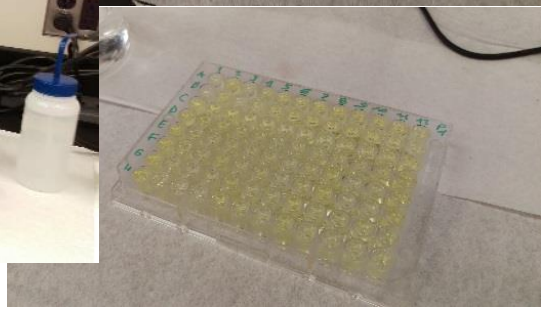
- A small portion of ground up faecal sample were mixed with methanol in a multivortex

Extraction

- After centrifugation in a centrifuge supernatant was collected and stored for further immunoassay







Enzyme-immunoassay

- The hormone (antigen) in the sample was bound to a specific antibody against this antigen and detected through enzymatic labelling

- Hormone concentration was measured using standard curve of the same hormone

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