Photo: 2016Aug6 Lamni

Relationships between spore feeding insects and wood decaying fungi

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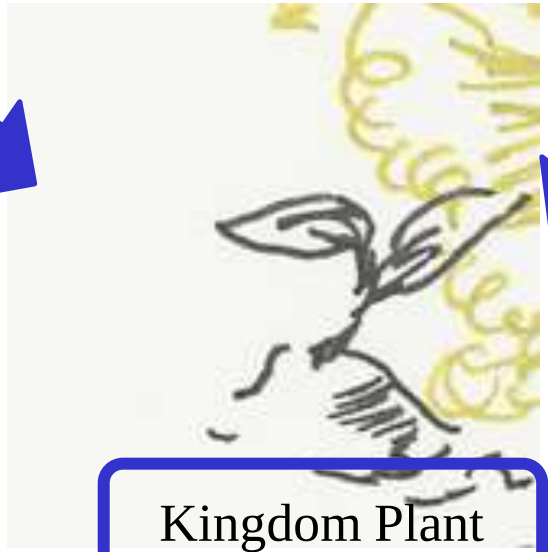
Symbiosis create and facilitate variable ecosystems in the world.

Kingdom Animal

Ambrosia beetles,
Leaf cutting ants

Kingdom Fungi

Pollination,
Seed dispersion



Kingdom Plant

Mycorrhyzal fungi

What I am talking about

- Community structure of mushroom feeding insects
- Spore dispersal strategies of ephemeral fungi and long lasting fungi
- Prediction of importance of entomophily for fungi

Hypothesis: Variable fungal fruit bodies have evolved under animal predation pressure, and parts of the food chains has changed into mutualism.

I mapped relationships between macro-fungi and fungivorous insect

Study sites

Northern Japan (42N, 141E, cold temperate) 1995
Central Japan (35N, 135E, warm temperate) 1991
South east Ethiopia (7N, 35E, subtropic) 1993-94

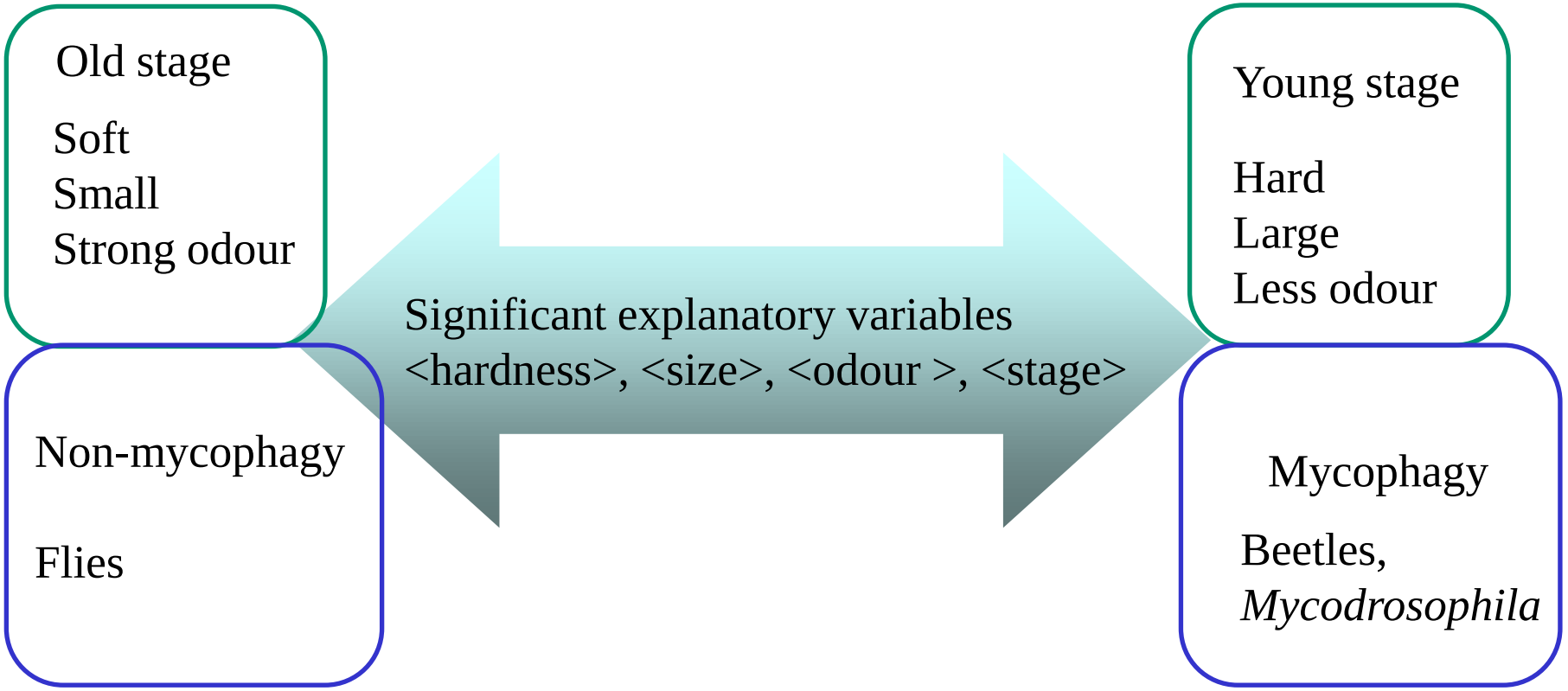
Insect assembly

- Ecological role in food web (fungivore, parasites, predator)
- Feeding type (hymenium, context, spore, not-specialized)
- Dependence on fungal resources (obligate, secondary, non mycophagy)

Fungal features

- Stability (size and longevity)
- Physical features (hyphal system, flesh type)
- Odour at ripe stage

Ordination of fungal species by canonical correspondence analysis revealed two clusters



Tuno, 1999, 2001

Among various insects, drosophiliids dominate in number and utilize wide range of fungal taxa

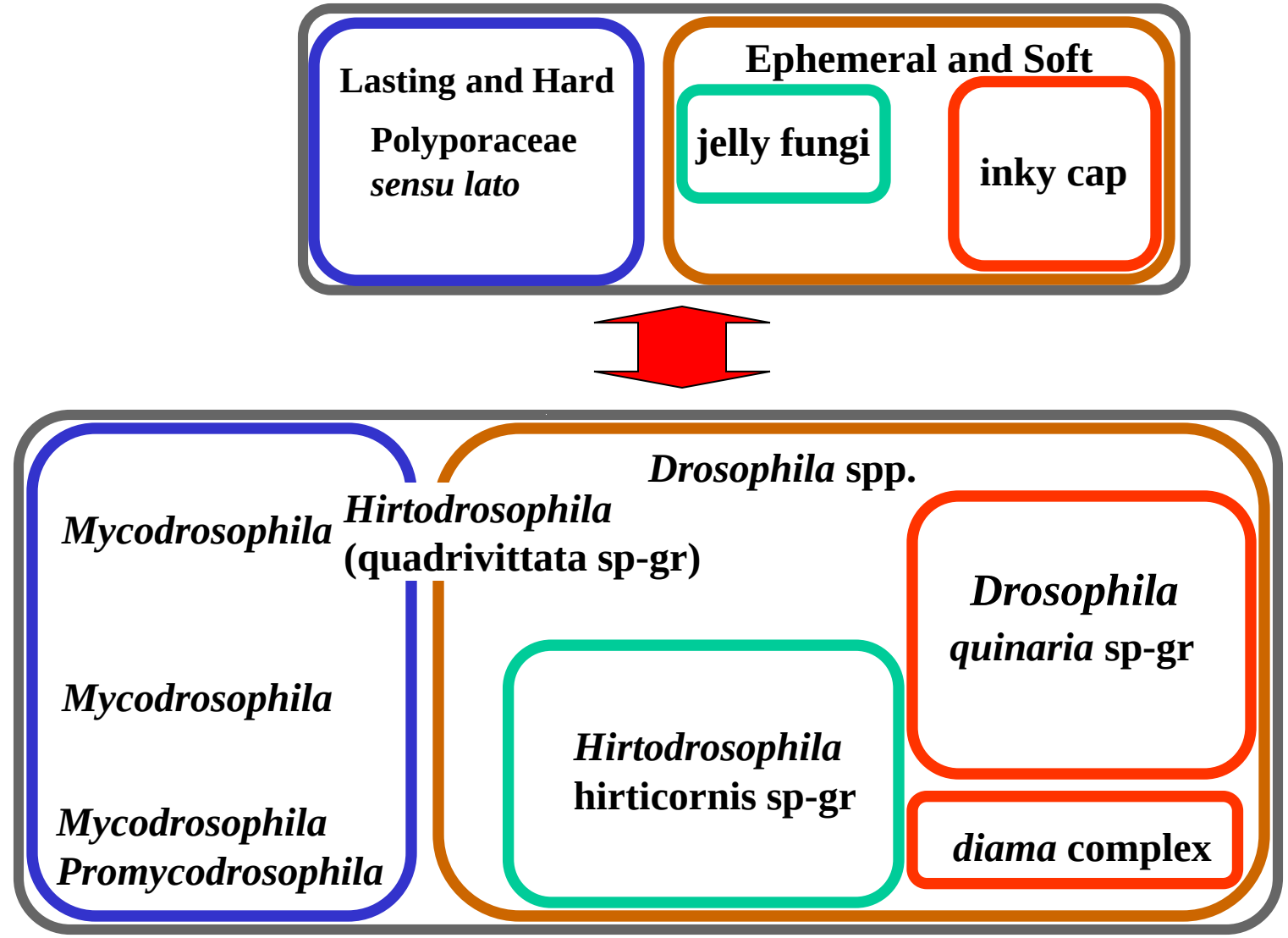
Drosophilids status

site	**SW-Ethiopia**	**Kyoto**	**Tomakomai**
proportion	**79%** (4390)	**36%** (2914)	**23%** (3417)

Composition of mycophagous drosophilid assemblages at 3 sites

genera	ratio	N of sp	ratio	N of sp	ratio	N of sp
Leucophenga	0.3%	2	0.5%	1		
Microdrosophila			0.3%	1		
Scaptomyza	0.0%	1	0.6%	1		
Scaptodrsophila	**1.8%**	**2**	**4.6%**	**1**		
Liodrsophila	0.4%	2	0.9%	2		
Drosophila	**3.1%**	**19**	**28.7%**	**13**	**5.8%**	**2**
Hirtodrosophila	**45.8%**	**5**	**8.6%**	**6**	**78.1%**	**6**
Mycodrosophila	**34.9%**	**21-25**	**55.2%**	**6**	**16.0%**	**5**
Dichaetophora	0.0%	1				
Zaprionus	0.0%	1				

Fungal resource for mycophagous drosophilid represent the same two clusters with several sub clusters



- ✓ Community structure of fungi and fungivorous insects with special reference on Drosophillids
- ☐ Spore dispersal patterns of ephemeral fungi and long lasting fungi
- ☐ Prediction of importance of entomophily for fungi.

Ephemeral and Soft

***Phallus indusiata* , Ethiopia**

Stink horns' general features

1. Strong odour
2. Ephemeral
3. Spores are in liquid (gleba)
4. believed Entomophily

Tuno 1998

Field Sampling *–to specify insect assembly*

Features of individual insects

-Exclusive feeding on liquid (gleba)

-Non-mycophagy

-Few adhesion of gleba on body

Features of insect assembly

-variable without fixed member

Spore germination experiment

Species	Sex	Number of speciemen	Number of spores in a rectur Mean±S.D.			Germination rate (n) at the 3rd days after observation started		at the 5th days	
D. lutescens	**f**	**2**	**35,840**	**±**	**27,955**	**0.79±0.03**	**(585)**	**--**	**--**
D. busckii	**f**	**2**	**102,400**	**±**	**74,218**	**0.87±0.06**	**(436)**	**0.89±0.04**	**(893)**
D. immigrans	**f**	**3**	**240,640**	**±**	**135,765**	**0.85±0.12**	**(1507)**	**0.99±0.02**	**(4219)**
D. immigrans	**m**	**8**	**152,000**	**±**	**102,596**	**0.87±0.08**	**(1521)**	**0.95±0.07**	**(1835)**
Muscidae spp.	**f**	**3**	**1,681,920**	**±**	**460,231**	**0.74±0.06**	**(526)**	**0.87±0.09**	**(236)**
Control						**0.81±0.03**	**(227)**	**0.95±0.03**	**(562)**

- ✓10^6-10^8 spores are consumed to be excreted
- ✓Digested spores germinate as much as control

Discussion

Stinkhorns

Strong **Odour**

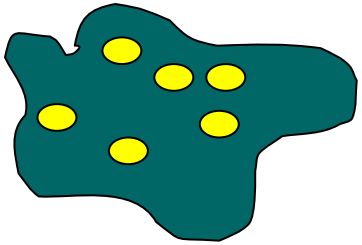
-mimicry fallen fruits chemically

Spores in liquid

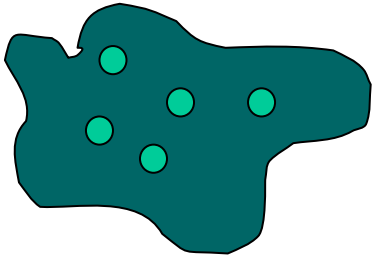
-mimicry fallen fruits physically

-Non-mycophagy

-Variable insect species in time and place



Bacteria /yeast in liquid medium



Spore in gleba

Ephemeral stinkhorns attract and recruit non-mycophagous insects by strong odour and accessible physical character.

heart rotting fungi,
G*anoderma applanatum*

- common species in Japan that produce perennial sporocarps .
- may yield tree cavities which are utilized as nests by a variety of animals, therefore it can facilitate species diversity.
- discharge spores which outer thick transparent substances coat brown-colored inner wall with projections.
- spores are under dormancy to germinate irregularly spending about one year (Aoshima 1954) .

Captured Insect assembly

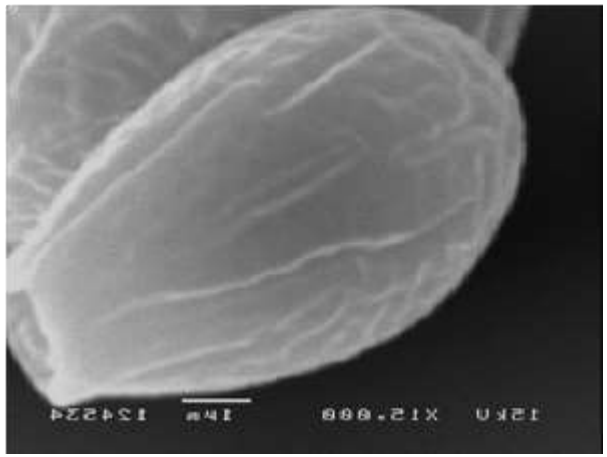
		developmental stage of sporocarp		
		Young	Mature	Old
Order	Family	66 days	27 days	90 days
Coleoptera	Cucujidae	0	1	1
	Discolomidae	0	5	0
	Phalacridae	0	1	0
	Scaphidiidae	4	2	2
Diptera	Cecidomyiida	0	1	0
	Sciaridae	0	1	0
	Drosophilidae	2	108	16
	Heleomyzidae	0	0	1
	Phoridae	0	2	0
Hymeno	(parasitoid)	0	2	0
Psocoptera	Caecillidae	0	2	0
Total number of individual		6	125	20

crop filled with spores

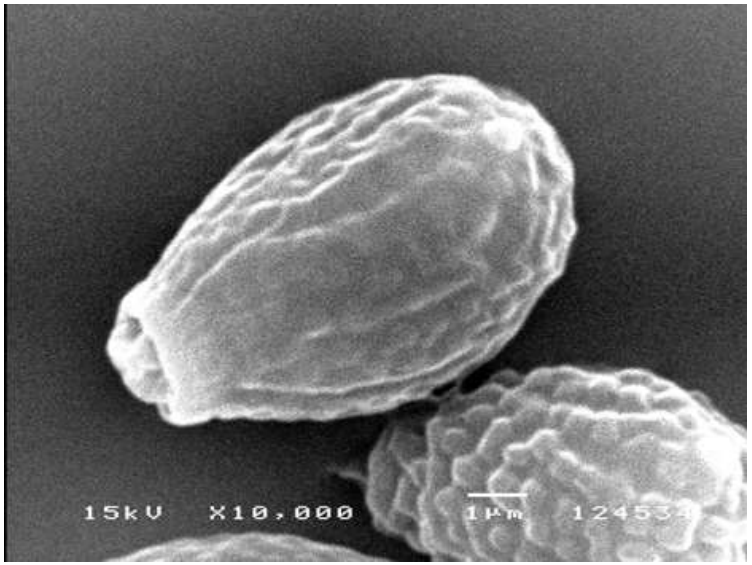
Species	1991 Jul.-Sep.	1996 Jul.	1996 Oct.-Nov
Mycodrosophila gratiosa	105	24	105
My. poecilogastra	3	23	225
My. erecta	0	0	7
My. japonica	0	0	4
My. planipalpis	0	0	2
My. shikokuana	0	0	4
My. takachihonis	0	0	1
Scaphisoma spp.	2	7	10
Others	15	0	9
Total	125	54	367

Spore discharging sporocarps attracts more insects, *Mycodrosophila* and *Scaphisoma* predominate in number

Mycodrosophila flies digest outer coating of _Ganoderma_ spores



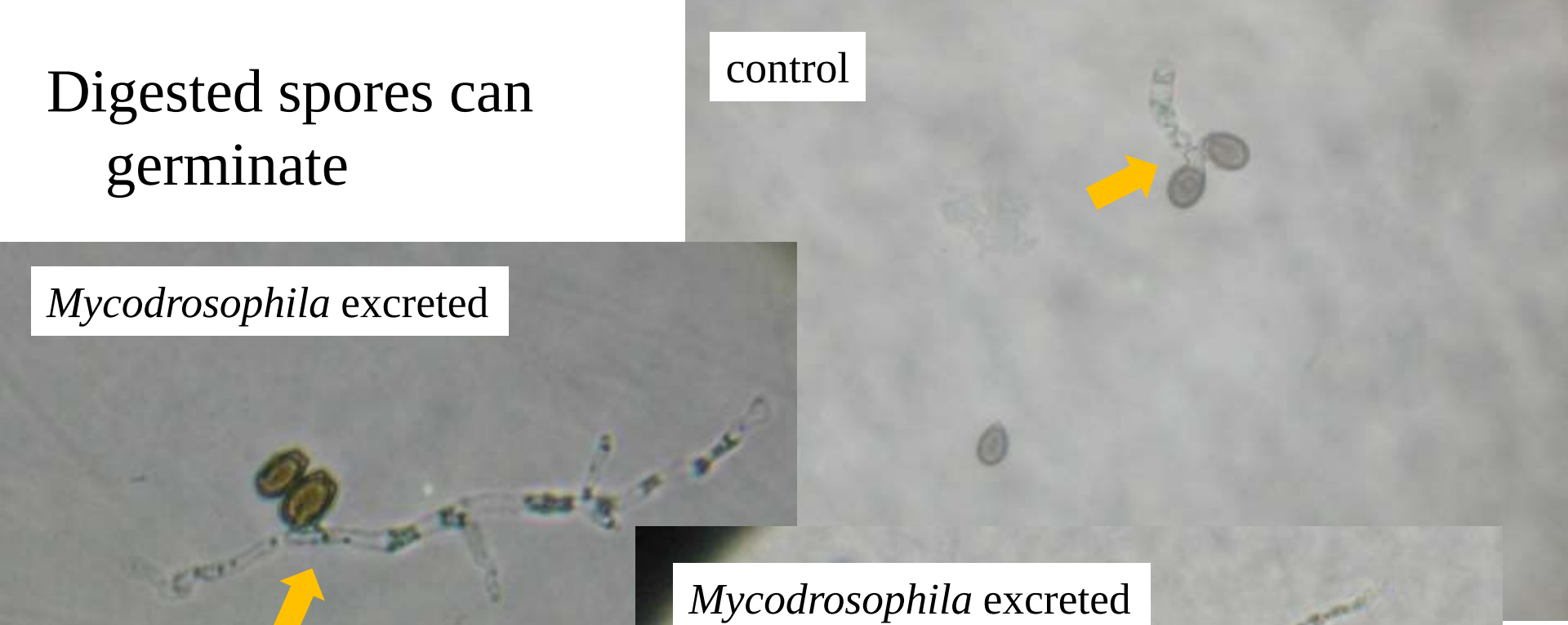
Spores in crop



Spores in rectum:
outer coating is removed

Digesting the coating

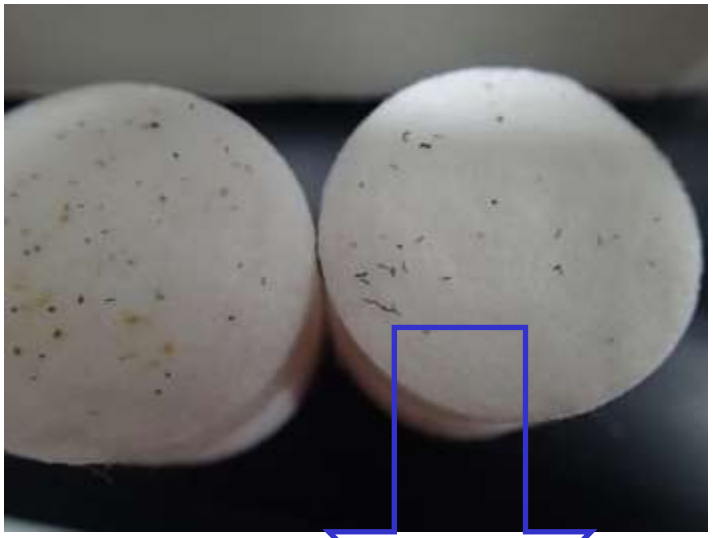
Digested spores can germinate

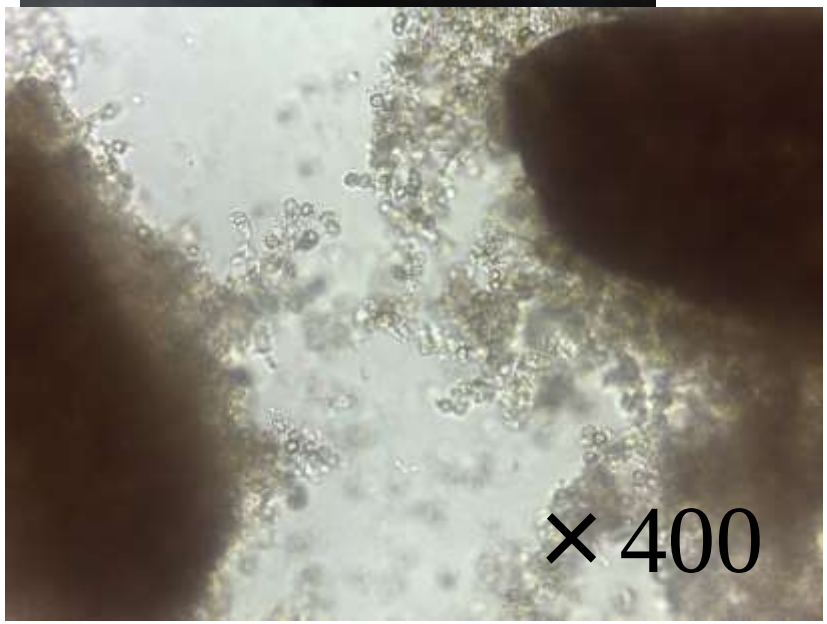


Photos: Ms. Natuki Shimotoku

Species	Sex	N	N of spores (Mean±S.E.× 10^4) in a Rectum	N of spores (Mean±S.E.× 10^4) on Body surface
Drosophilidae				
My. gratiosa	**f**	**4**	**4.7 ± 2.2**	**1.5 ± 1.2**
	m	**4**	**3.6 ± 3.6**	**1.1 ± 0.7**
My. poecilogastra	**f**	**6**	**2.5 ± 1.9**	**0.3 ± 0.4**
	m	**11**	**1.4 ± 0.6**	**0.9 ± 0.8**
Scaphidiidae				
***Scaphisoma* sp.**	**f**	**3**	**1.5 ± 0.8**	**0.0**

Spores are excreted or dropped by brushing at their resting places

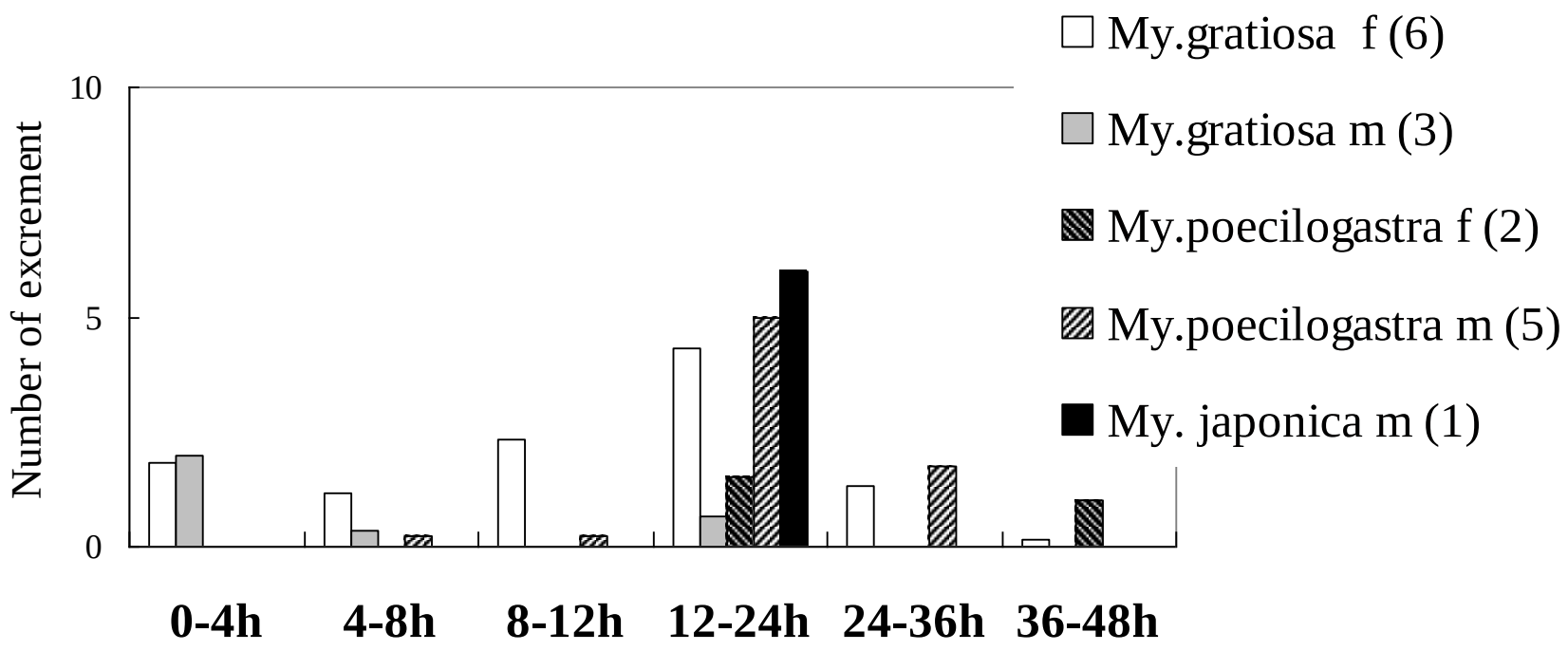




Drosophillids drop bars of lengths of their tibiae (part of legs)

mass of spores and mold by body brushing

When do flies excrete?



Periodical frequency of *Mycodrosophila* excretion after feeding on *Ganoderma applanata* sporocarps

Spores are excreted one or two days later

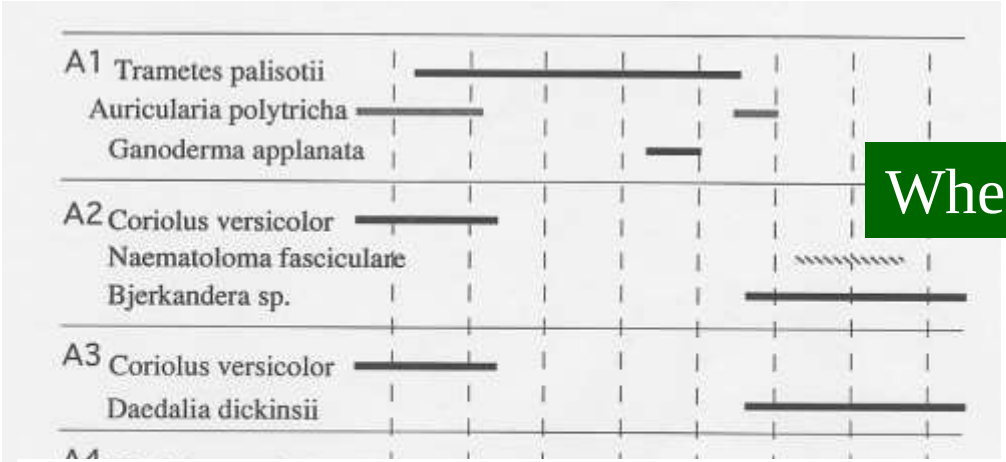
Where do flies go after feeding?

I captured flies from different sporocarps of *Daedalea serialis* to examine their digestive tracts

Species	with the spores of *G. applanata*	Total number
My. gratiosa	2	2
My. poecilogastra	3	41
My. erecta	0	1

Direct evidence:

Mycodrosophila move among logs harboring bracket fungi

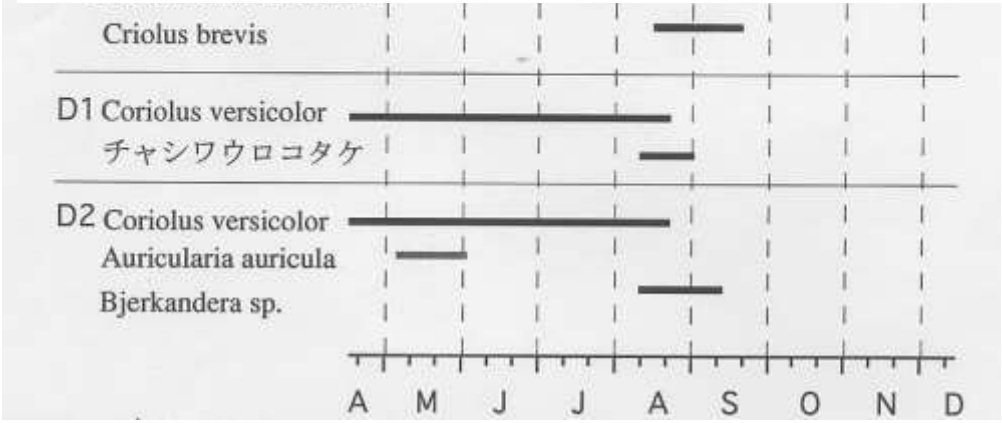


Where do flies bring spores?

Circumstantial evidence

Most logs harbor plural fungal species and they sporulate in different timings

“Spore feeders move among decaying woods



Now we can make direct evidence by molecular analysis

fungal phenology per logs in the botanical garden, 1991, Kyoto

- *Mycodrosophila* and *Scaphisoma* exclusively feed on spores of wood rotting fungal species.

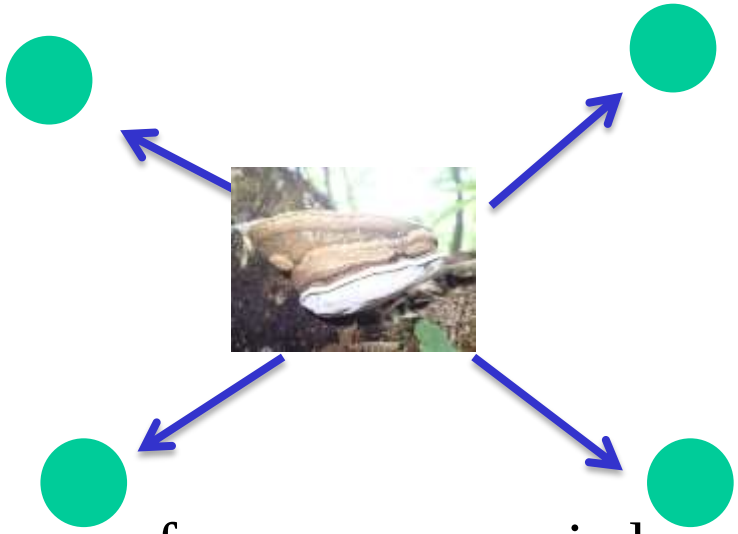
- It is not species-specific relationships but guild-guild relationships

- ✓ Community structure of fungi and fungivorous insects with special reference on Drosophillids
- ✓ Spore dispersal of ephemeral fungi and long lasting fungi

Next question is

- ☐ Is entomophily important for wood rotting fungi?

Entomophily



mass of spores are carried without changing spore density, or size

Physical dispersion

- More distant, less spore, The bigger, the shorter

(Li, 2005; Galante *et al*. 2011)

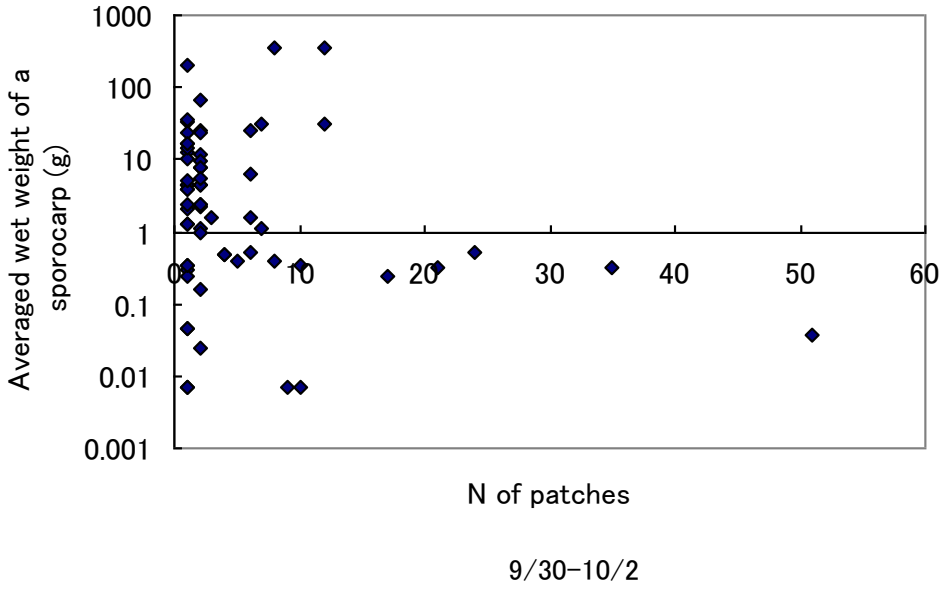
advantages to recruit animal as vector

Spore dispersal of fungi

Hypotheses

Dispersal by wind: Random-----Abundant resource
Dispersal by animal: Directional ------Rare resource

sporocarp weight, shape, frequency, in 100 quadrat (20m sq.) in a cool temperate forest



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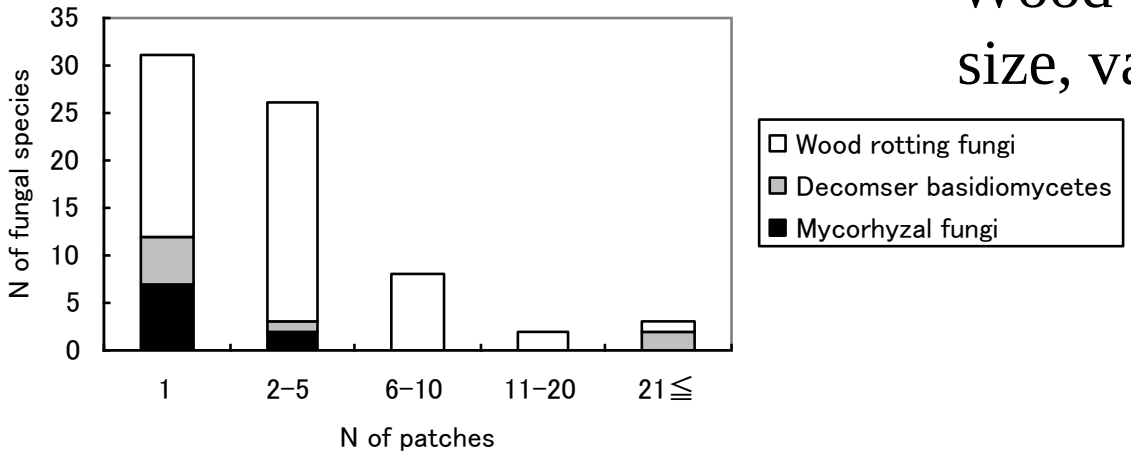


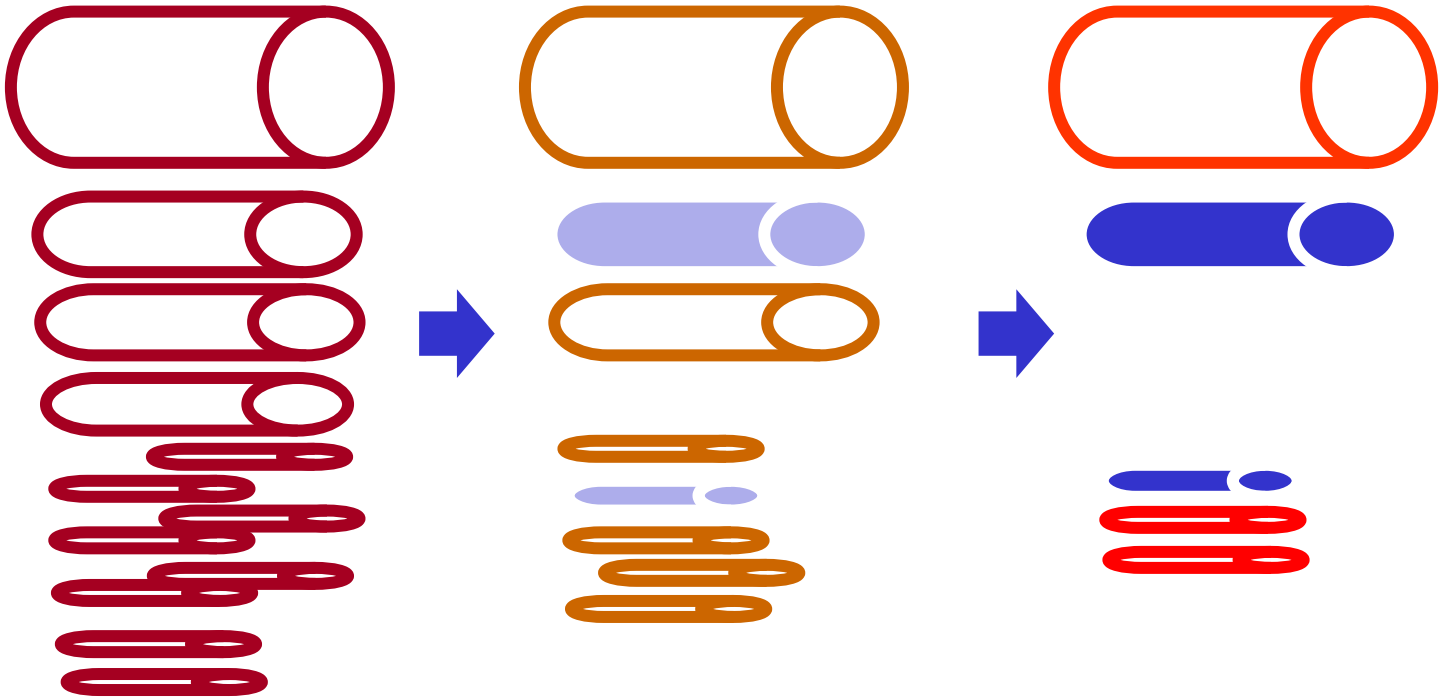
Decomposer basidiomycetes: small cap with thin stipe (5cm), abundant

Mycorrhizal fungi: large, with tick stipe (5cm), rare

Wood wotting fungi: variable size, variable frequency

Wood rotting fungi which need rare resources (size, decaying stage) will favor recruiting animal with directional dispersion

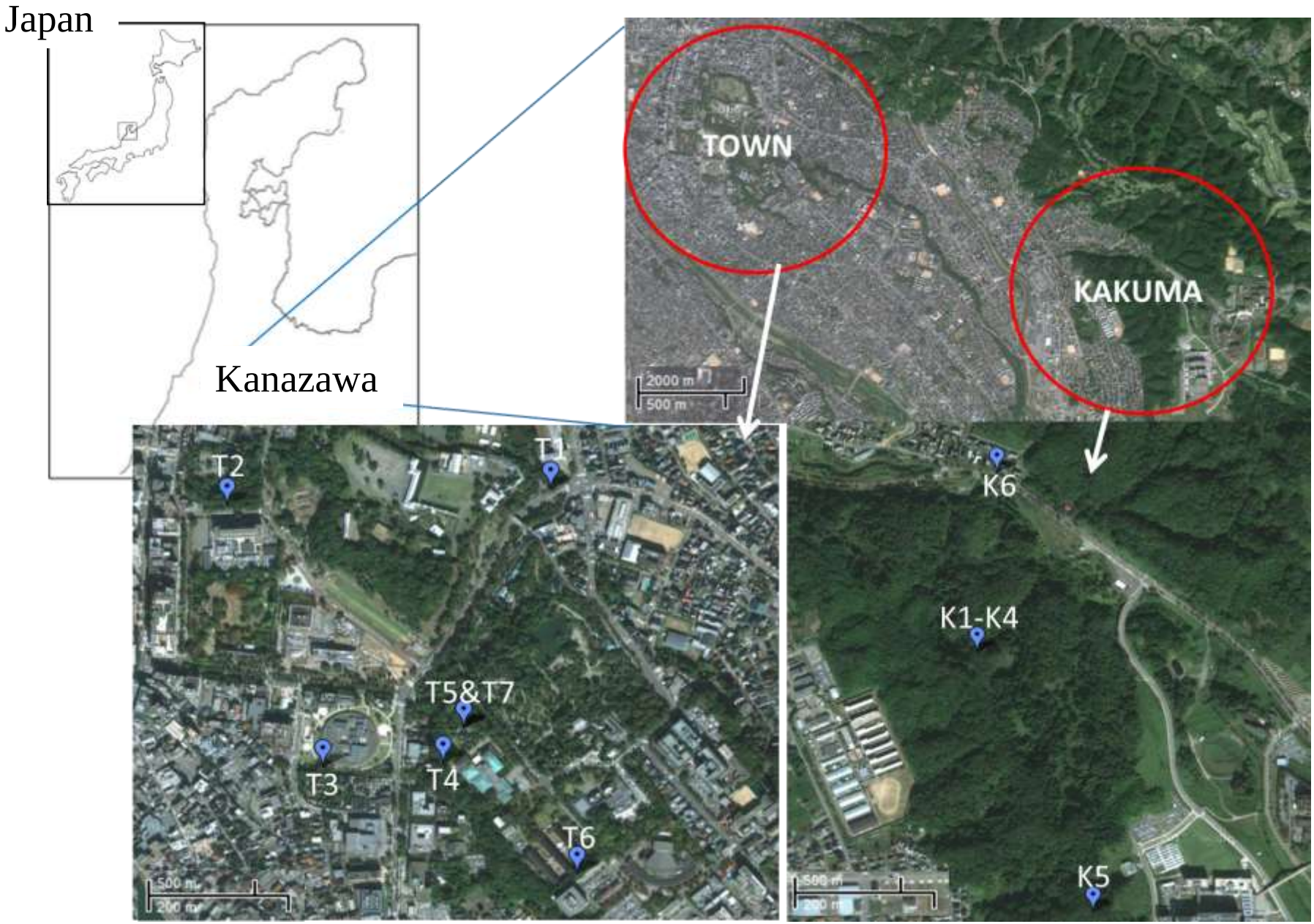
Modular structure of tree: larger CWD is rare, smaller CWD is more

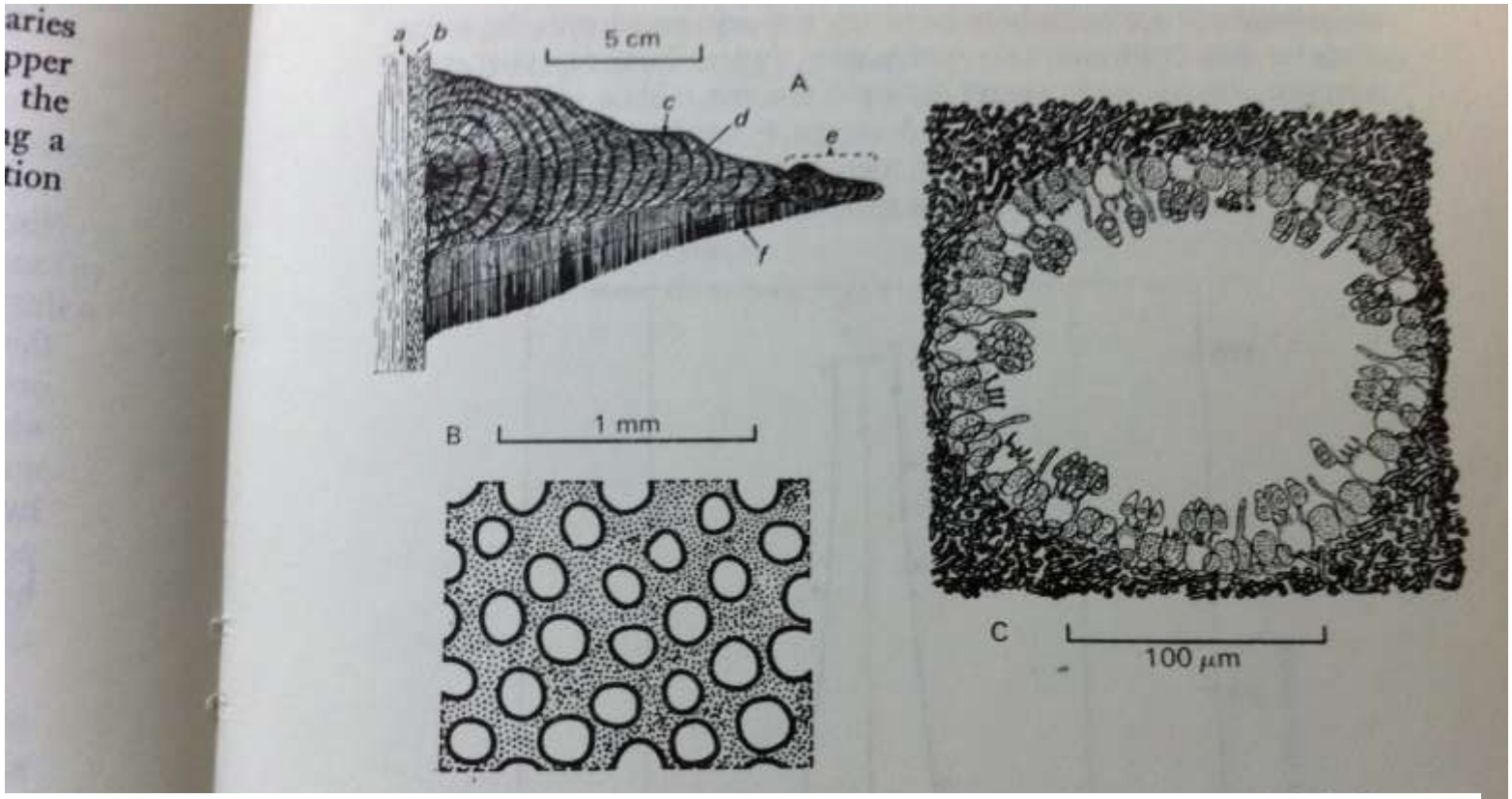


Positive priority effects of two fungivorous beetles on *Ganoderma applanatum*（Jacobsen et al 2015）

---certain fungi will colonize after certain fungi or will be inoculated by fungivorous insects

G. applanatum location in Kanazawa city





Ganoderma applanatum

(A) Vertical section of a fruit-body growing on an ash tree

(B) Horizontal section of fruit-body at the level of hymenium tubes

(C) Details of a single hymenial pore

From Ingold TC, 1971, Fungal Spores: Their Liberation and Dispersal

Without spore feeding insects, abundant spores may stack to the hymenial pores to disturb spore discharge into the air

Feeding habits in dipteran adults

Family	n	spore-feeding	ratio (%)
Drosophilidae	134	109	81.3
Phoridae	36	1	2.78
Sphaeroceridae	17	0	0
Mycetophilidae	15	0	0
Cecidomyiidae	13	0	0
Sciaridae	8	0	0
Heleomyzidae	8	0	0
Chloropidae	7	0	0
Ceratopogonidae	4	0	0
Chironomidae	2	0	0
Milichiidae	1	0	0
Dolichopodidae	1	0	0
Platypezidae	1	0	0
Clusiidae	1	0	0

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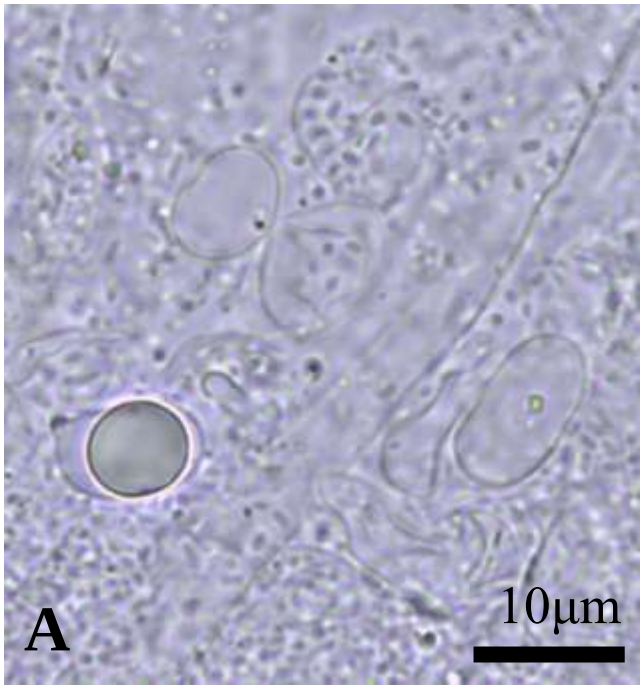
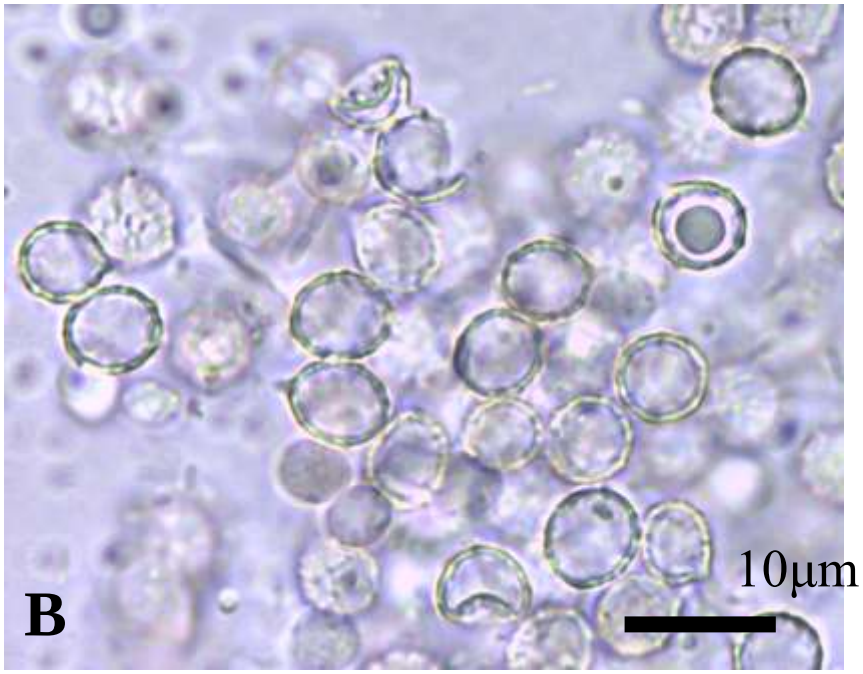
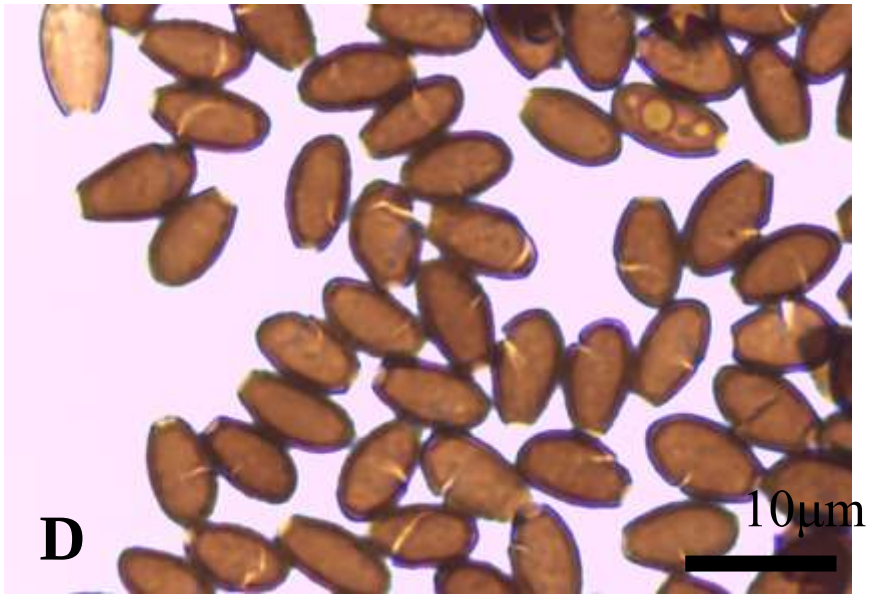
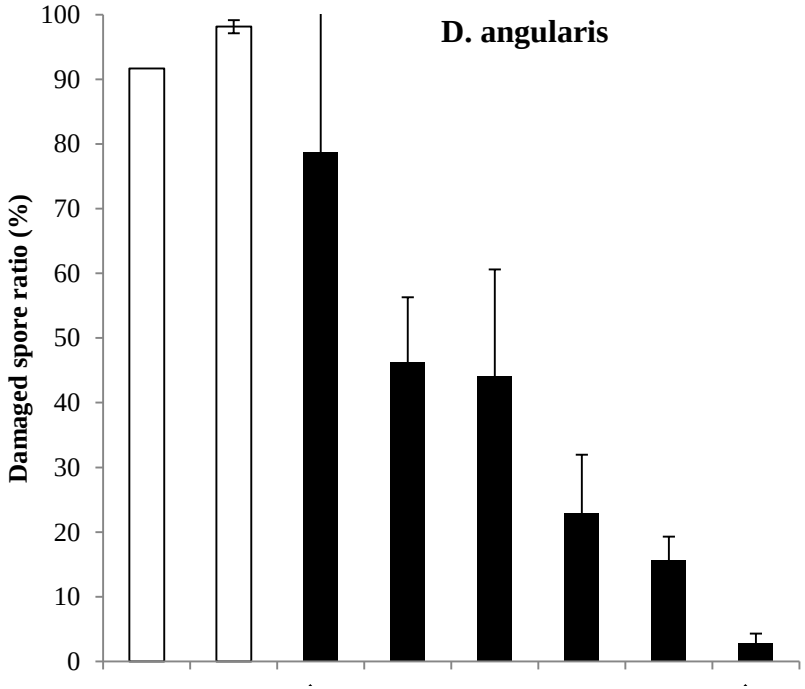


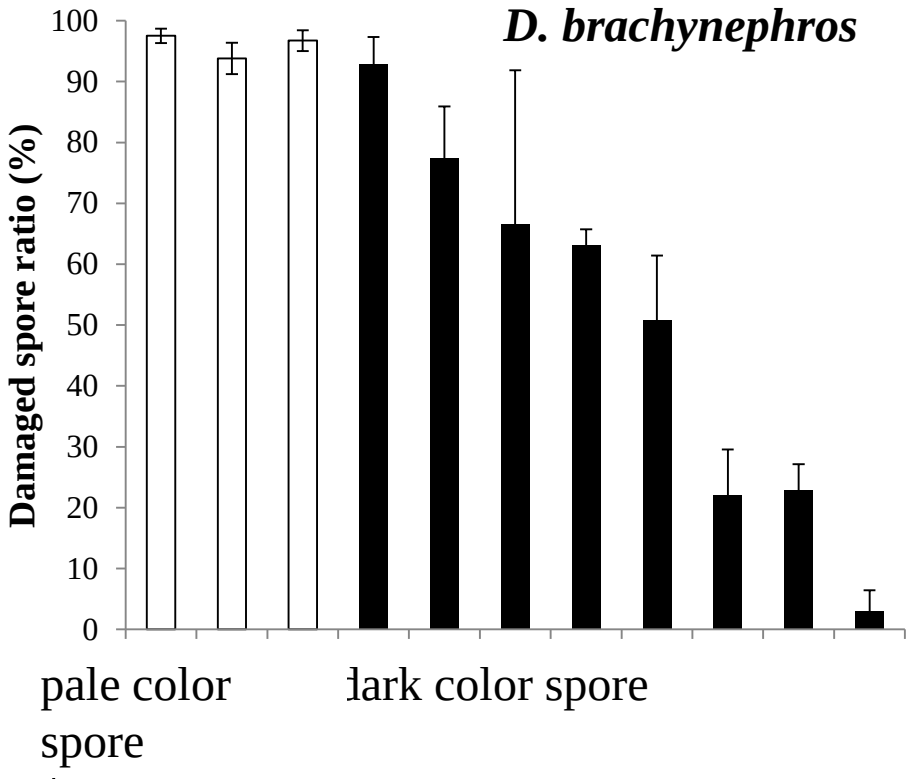




Spore digested by *Drosophila brachynephros* photo by Ms. Mio Kobayashi

Destroyed ratio of basidiospores by digestion by mycophagous *Drosophila*





Pale colored spores are damaged heavily while dark colored spores survived

data: Ms. Mio Kobayashi 2015

Importance of entomophily for wood rotting fungi may be predicted by

- Rarity of resource for fungi (size of CWD and its decaying stages in fungal succession)

- Characteristics of spore and hymenophore


Thank you Dmitry and organizers for giving us opportunities to share our interests
All of you are welcome to Japan, anytime!