Interaction in a two-way crossed ANOVA

Artificial examples to help interpretation

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Context: Two-way crossed analysis of variance (ANOVA) with replication

This document deals with interaction in ANOVA. Specifically, the examples are those of an analysis of variance with two crossed (i.e. independent, as opposed to hierarchical or nested) factors, with more than one observation in all combinations of levels of the two factors.

This model tests whether there are differences in means between the levels of the main factors, and whether there is an interaction.

The following slides present an example of all the cases where at least one of the tests (main factor or interaction) rejects the null hypothesis. (In the first case shown, neither the main factors nor the intraction are significant.)

Interaction : definition

An interaction is the influence that the state of one factor has on the **effect** of the other factor on the response variable.

An interaction occurs when the effect of one factor changes according to the level of the other factor.

Example of an interaction: the increase in effect of an anti-hypertensive drug given at a higher dose is greater in smokers than in non-smokers.

Interaction – example

The following, artificial situations have been constructed from an example presented by Sokal and Rohlf (1995) in their *Biometry* textbook.

The experiment consisted of feeding fresh or rancid lard to male or female rats. In the example of Sokal and Rohlf, the total consumption (in g) of lard over 73 days by 12 rats aged 30 to 34 days at the start of the experiment was measured. This consumption is the response variable, the two factors being the freshness of the lard and the sex of the rats.

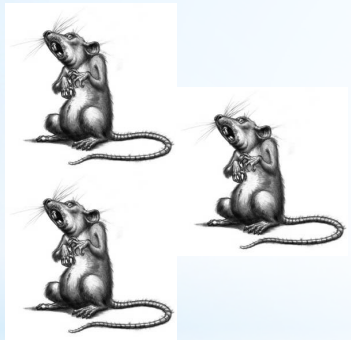
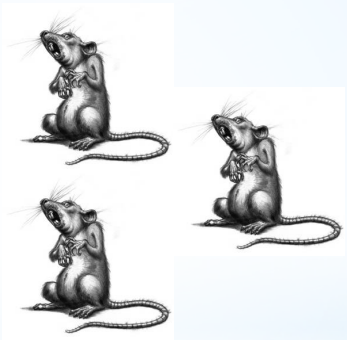
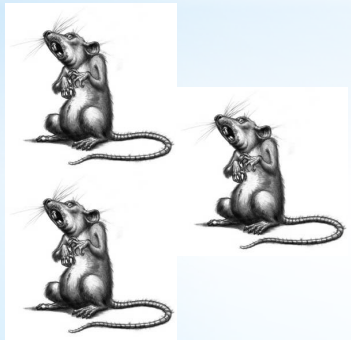
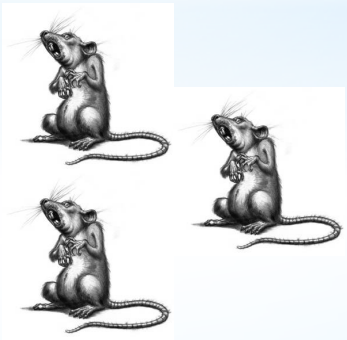
Sokal, R.R., & F. J. Rohlf. 1995. *Biometry – The principles and practice of statistics in biological research. 3rd Edition*. W. H. Freeman, New York.

Interaction – example

Response variable *y*: lard consumption (g)

Factor A: sex of the rats (male – female)

Factor B: freshness of lard (fresh or rancid)

	Fresh	Rancid
Male		
Female		

3 replicate rats per cell

Interaction – example

How would you interpret a significant interaction?

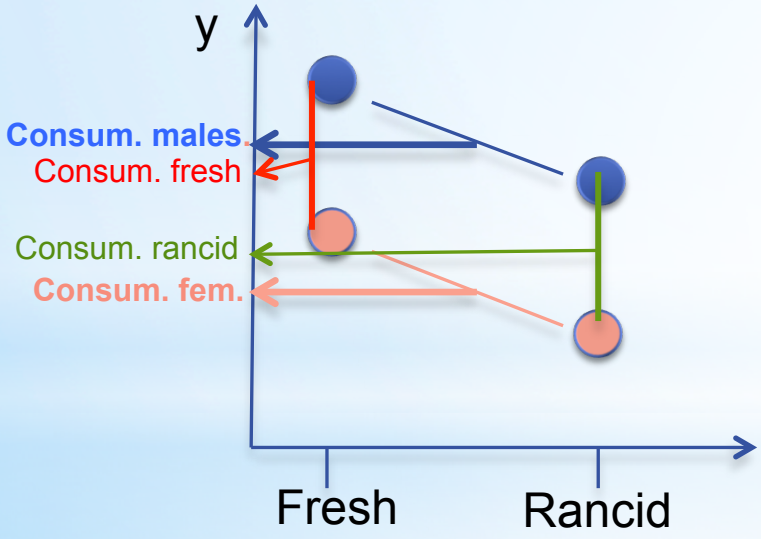
Interaction – example

How would you interpret a significant interaction?

Preference between fresh or rancid lard depends on the sex!

Interaction – example

The following slides each show two or three interaction plots. Each plot illustrates a different outcome of the experiment.



In the diagram, the ordinate represents the value of the response variable, the abscissa represents the lard freshness and the dots represent the sex of the rats.

Interpretation is done by projecting the factors (combinations of levels) onto the ordinate.

Interaction – illustration

1. No interaction

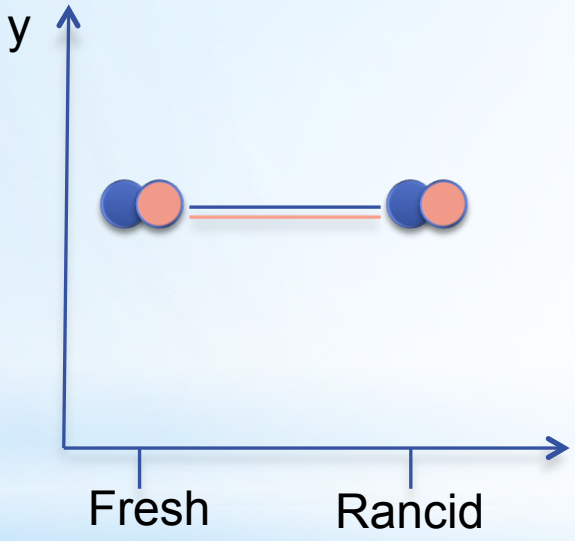
y = quantity of lard eaten by the rats (response variable)

= males

= females

n.s. = not significant ($p > 0.05$)

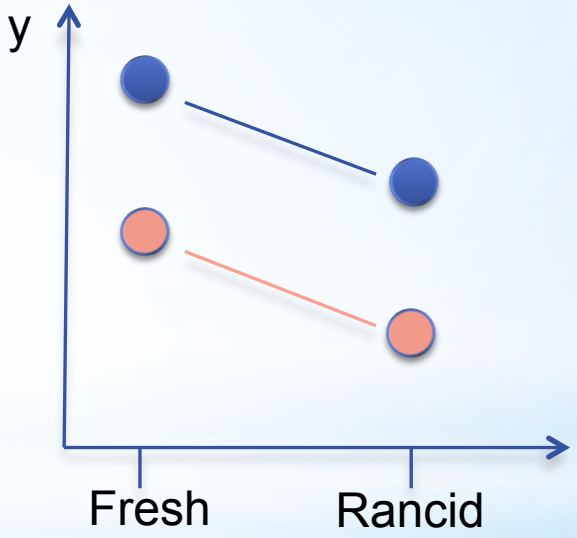
*** = significant ($p \leq 0.05$)



Freshness : n.s.

Sex: n.s.

Interaction: n.s.



Freshness : ***

Sex: ***

Interaction: n.s.

Interaction – illustration

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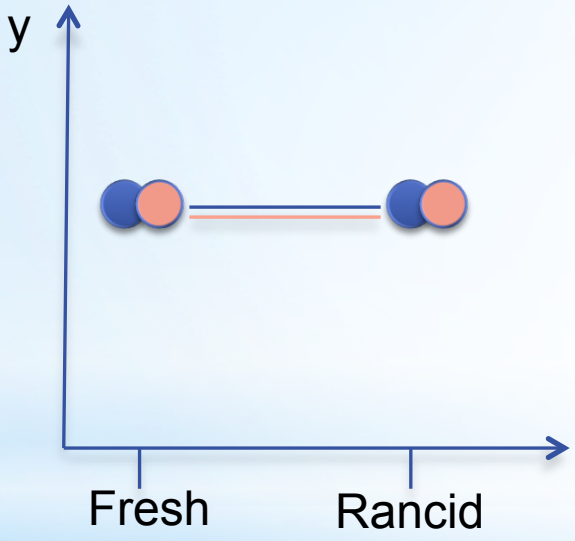
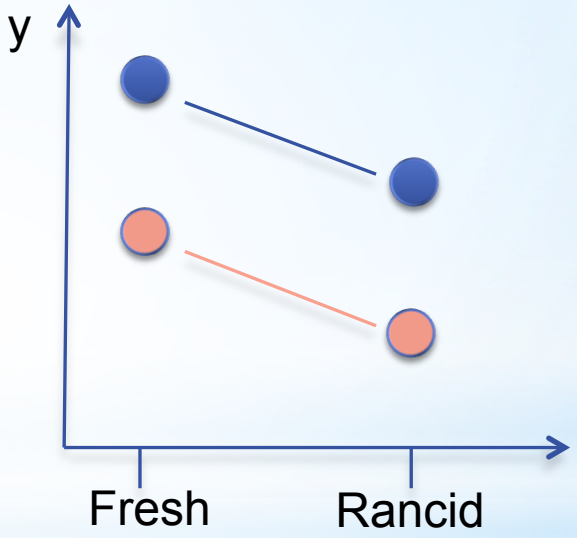


Males eat the same amount of lard as females.
Rats eat the same amount of fresh lard as rancid lard.
The lines are superimposed (no interaction).



Males eat more lard than females.
Rats eat more fresh lard than rancid lard.
The lines are parallel (no interaction).

Interaction – illustration

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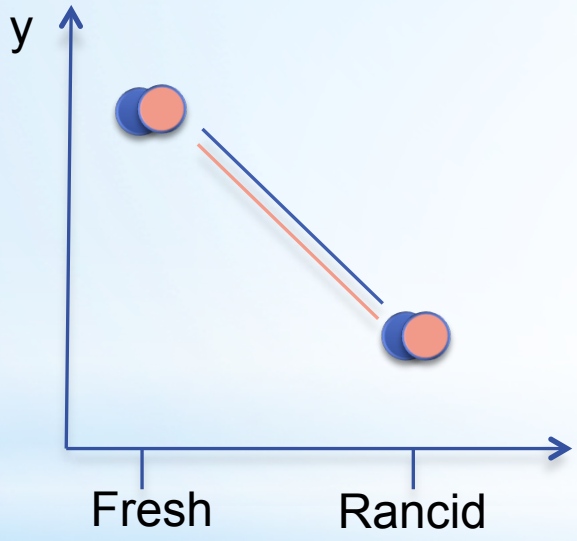
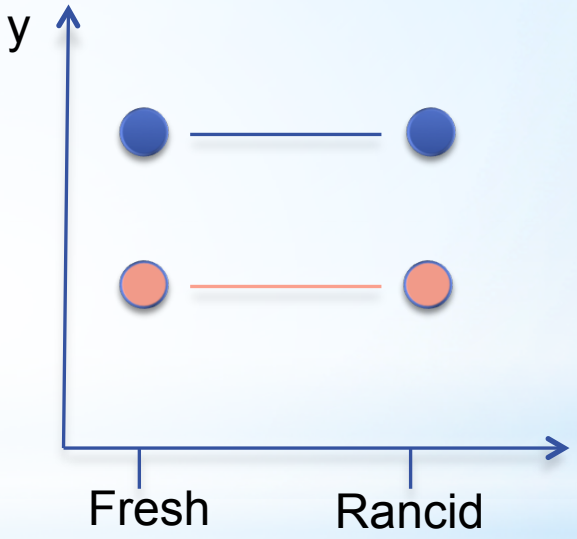


Freshness : ***

Sex: n.s.

Interaction: n.s.



Freshness : n.s.

Sex: ***

Interaction: n.s.

Interaction – illustration

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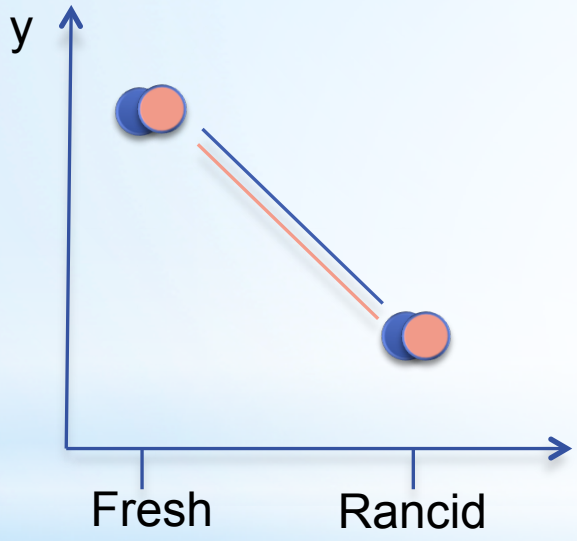

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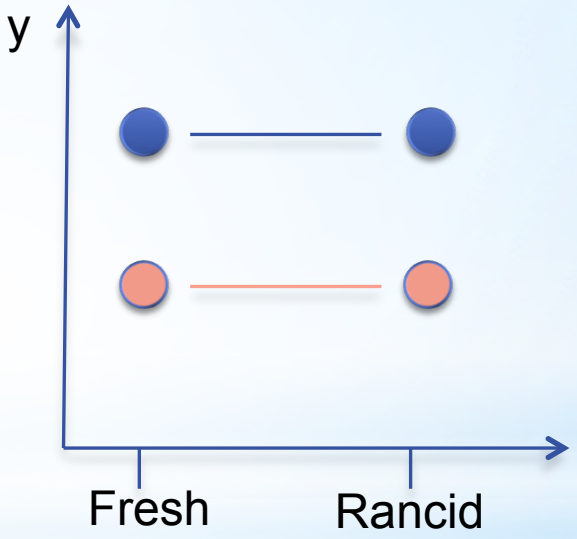

Males eat more lard than females.
Rats eat the same amount of fresh lard as rancid lard.
The lines are parallel (no interaction).

Interaction – illustration

2. Significant interaction

y = quantity of lard eaten by the rats (response variable)

= males

= females

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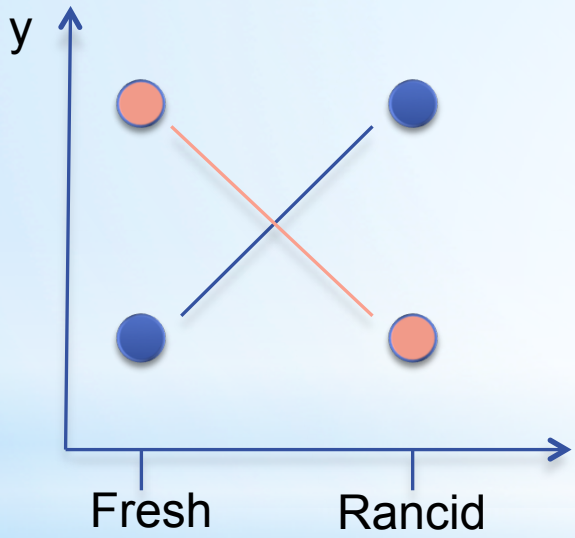
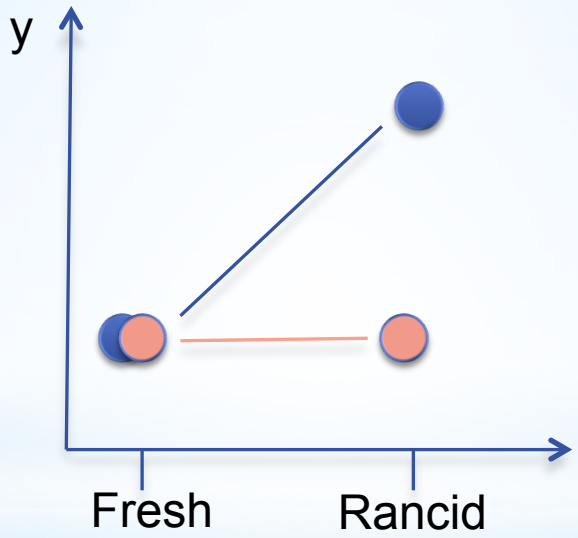
*** = significant ($p \leq 0.05$)



Freshness : n.s.

Sex: n.s.

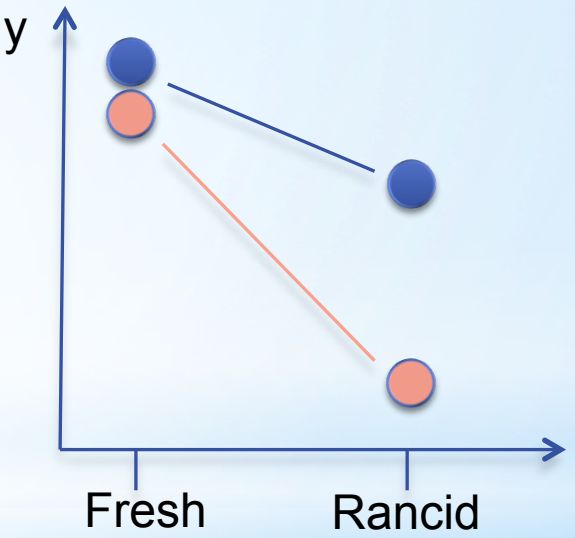
Interaction: ***



Freshness : ***

Sex: ***

Interaction: ***



Freshness : ***

Sex: ***

Interaction: ***

Interaction – illustration

2. Significant interaction

y = quantity of lard eaten by the rats (response variable)

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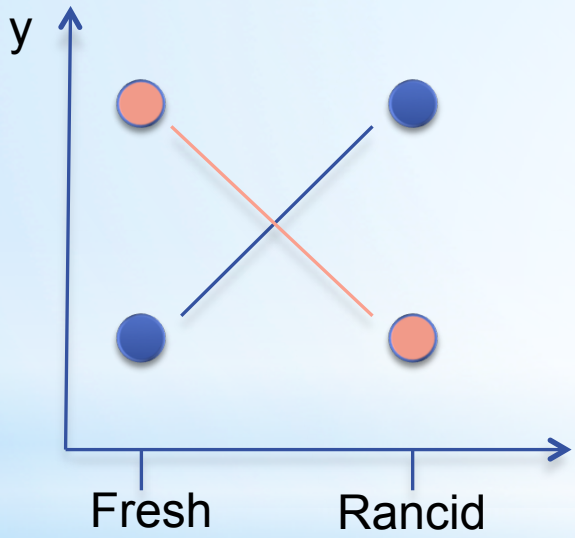
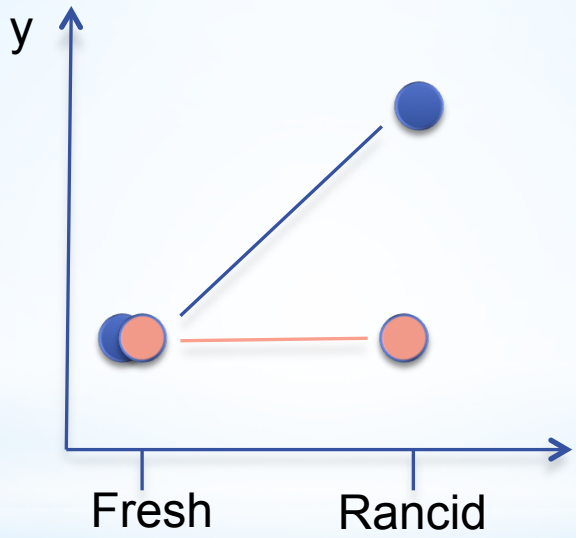
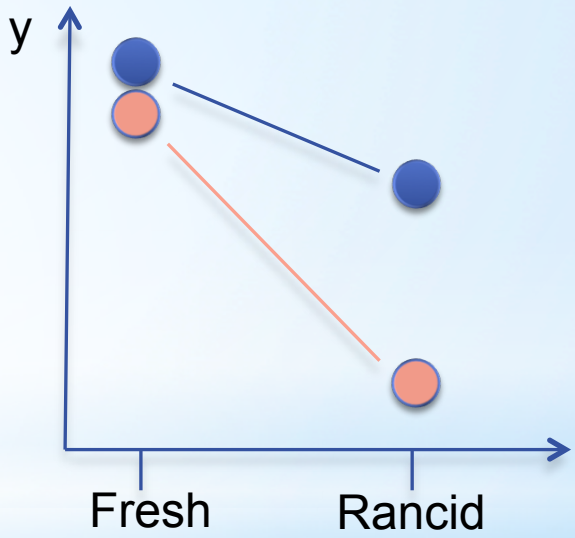


Males eat the same amount of lard as females.
Rats eat the same amount of fresh lard as rancid lard.
The lines are crossed: males prefer rancid, females prefer fresh.
Interaction.



Males eat more lard than females.
Rats eat less fresh lard than rancid lard.
Non-parallel lines: males prefer rancid, females are indifferent to freshness.
Interaction.



Males eat more lard than females.
Rats eat more fresh lard than rancid lard.
Non-parallel lines: the preference of males for fresh lard is less marked than for females.
Interaction.

Interaction – illustration

2. Significant interaction

y = quantity of lard eaten by the rats (response variable)

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= females

n.s. = not significant ($p > 0.05$)

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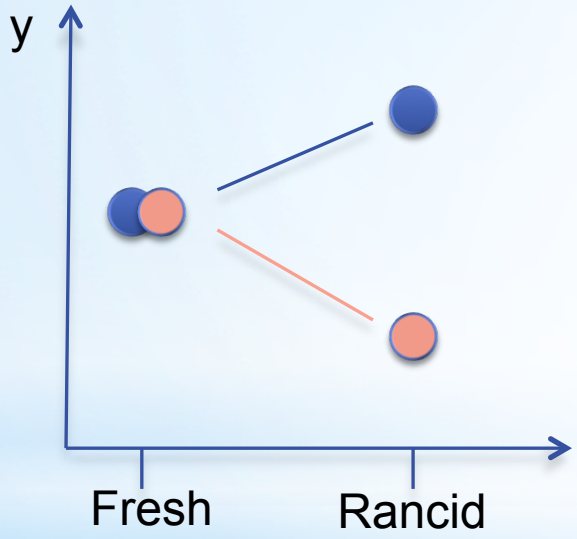
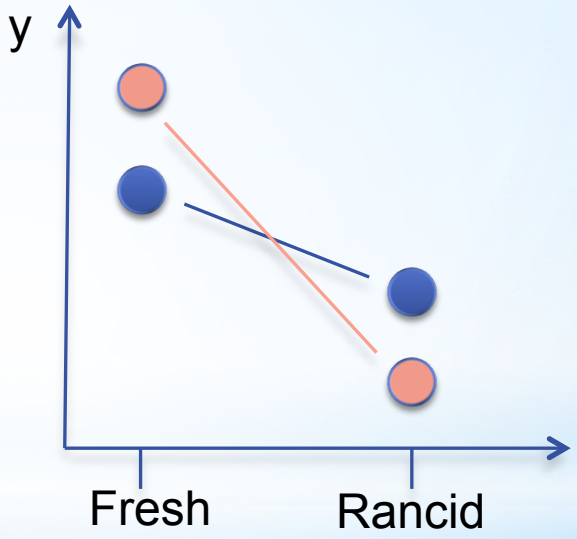


Freshness : n.s.

Sex: ***

Interaction: ***



Freshness : ***

Sex: n.s.

Interaction: ***

Interaction – illustration

2. Significant interaction

y = quantity of lard eaten by the rats (response variable)

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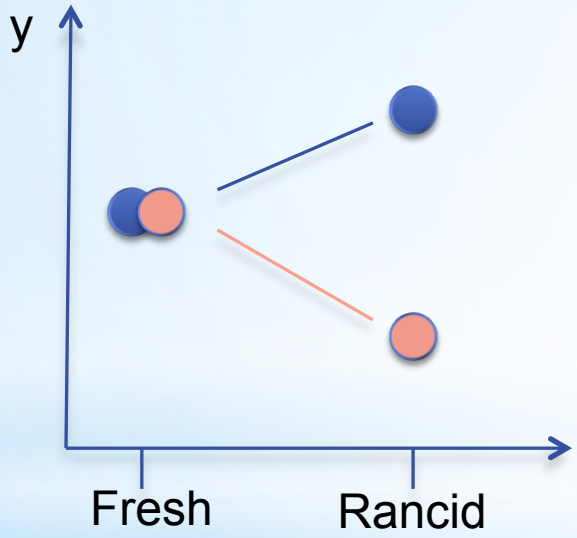
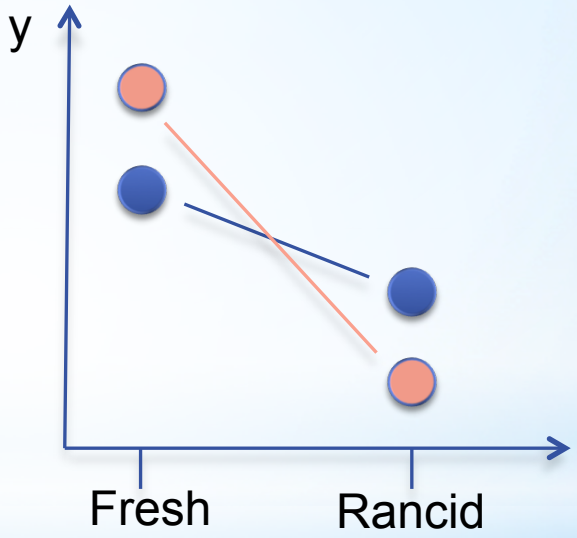


Males eat more lard than females.
Rats eat the same amount of fresh lard as rancid lard.
Non-parallel lines: males prefer rancid lard, females prefer fresh.
Interaction.



Males eat the same amount of lard as females.
Rats eat more fresh lard than rancid lard.
The lines are crossed: the preference of males for fresh lard is less marked than for females.
Interaction.

Interaction – an important consequence

In the presence of a significant interaction, the main effects cannot be interpreted globally.

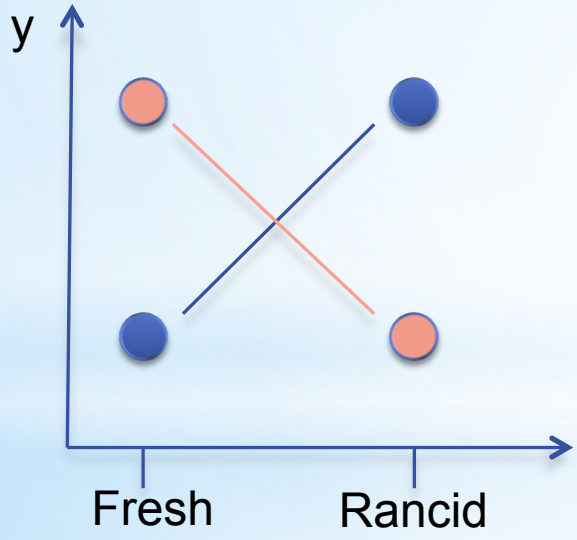
Why?

Because the main effect of one factor varies according to the state of the other.

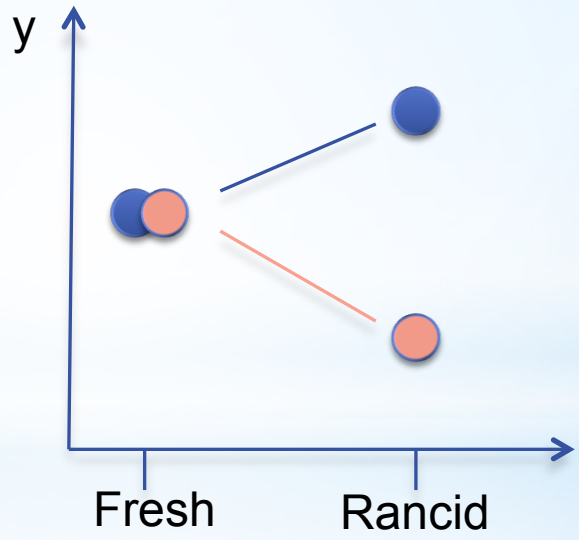
In our rats, when the interaction is significant, nothing can be said about the preference of fresh or rancid lard *without taking into account the sex of the animal!*

Interaction – an important consequence

In particular, when the interaction is significant, nothing can be deduced from the fact that one of the main factors is not significant.



Neither freshness nor sex significant



Sex significant, freshness n.s.

Global tests cannot be interpreted!

Interaction – an important consequence

Therefore, when the interaction is significant, the analysis of each factor should be repeated, limiting it to one class of the other factor at a time.

In the rat example, a significant interaction can therefore lead to up to four additional analyses:

- effect of freshness on male rats alone
- effect of freshness on female rats alone
- effect of sex for fresh lard alone
- effect of sex for rancid lard alone

The context of the study sometimes allows the number of additional analyses to be limited to those that are the most relevant.

Interaction – one last point

A significant interaction between two crossed factors always gives rise to two mathematically valid interpretations, although the context often favours one of the two. For the example of the rats:

Preference between fresh or rancid lard depends on the sex.

The amount of lard consumed by males or females depends on the freshness of the lard.