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Southern transect resurvey V.2

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We use this protocol and it's working

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Abstract

This protocol details the Ponisio Lab's collecting protocol for the 2022 Moldenke re-survey of the southern transect. Each site consists of two 900m long transects, marked every 100m with flagging tape and a nail. Each transect has three 10m² vegetation plots set ~10 m off at the beginning, middle and end of each transect. At some sites this placement of vegetation plots was not possible due to terrain so site maps/GPS points should be referenced. During a site visit passive traps are placed on one transect, and active netting and vegetation surveys are done on the other transect. The activities associated with each transect alternate every site visit.

Troubleshooting

Field station prep

1 Prior to collection, it is important to make sure that the following preparations are made:

Shared sampling box:

- GPS x2
- spare batteries
- flagging tape
- pin flags
- spare sharpies, microns etc
- transect tapes
- orange cones

Shared collection equipment/consumables:

- Freezeproof snap caps (netting)
- Vials for traps (labeled for pans)
- 70% ethanol jug
- 10% bleach jug
- Dry ice in Yeti (in the car)
- Freezer packs in Yeti (in the car)
- Quart freezer ziplocks for tubes + misc
- Gallon freezer ziplocks
- Freezer boxes
- Bleach bottle
- Bleach spray bottle (x3)
- Ethanol spray bottle (x3)

Personal sampling box + net:

- net
- stopwatch
- insulated fanny pack
- ice pack (frozen)
- butterfly box + butterfly envelopes
- kestral
- flat tweezer and pointy tweezer
- 2x thin sharpie(s) and 2x microns(s)
- plant guide
- orange vest
- walkie talkie

Pan/vane trap box:

- 5+ vane traps
- 30 pan traps sets (10 each of blue, yellow, white) + extra

- 3, 1L Nalgenes
- blue soap bottles
- ethanol squirt bottle * 2 (one for 70% ethanol)
- strainers
- scissors
- At field station: extra pans and traps

Communal camping supplies:

- solar panels + basecamp battery
- laptop in case + charger for data entry
- folding table
- shade cover
- cooking supplies
- water holders x2
- camp shower
- first aid kit (leave in van always)
- plyers (for cholla)
- mallet (for tents and rebar)

Datasheets:

- Weather Net
- Weather Traps
- Specimen
- Plant Quad
- Trap labels
- Unknown plants
- Packed in field desks *2

Label box:

- Colored tape for labeling boxes (green!)
- Lid label tape
- Thin tipped sharpie

Electronics:

- lab laptop and field case

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To prep before leaving to go sampling (also see checklists**):**

1. Freeze ice packs
2. Go over all electronic equipment and check batteries
3. Charge laptop(s) battery
4. Charge basecamp battery(s)
5. Charge AA rechargeable batteries
6. Check over traps for cracks
7. Buy dry ice

8. Assemble consumables (vials, ethanol)
9. FIll up giant water container for pan taps + other water needs
10. Check over individual sampling supply boxes
11. Assemble datasheets
12. Wash net bags with soap and water to remove pollen/bleach residue

Optional:

- Enough pan/vane trap tubes can be are pre-filled with ethanol and separated into individual bags for at least the next day, but often it is more efficient to do a lot of these at once. Fill the tube with ethanol, cap it, and put it in a ziploc bag. Fill the ziploc bag with 20 vials (enough for one subsite, plus 5 extras in case of over-filled pans). Add one eppendorf filled with 1mL of dish soap to each bag for pan trap setup as well.

Site Setup

3 Supplies:

- transect tape
- white pin flags
- flagging tape
- silver sharpie
- GPS
- compass
- spray painted rebar stakes
- mallet

Pollinator sampling transect: Each site has two 900 m transects. Find the end of each transect on the garmin. There may be a nail marker (installed 2020). Place a flag, label it 0. Orient the compass toward the end of the transect if it is not obvious (i.e., some are along a road or trail). Use the transect tape to measure out 100 m. Place a flag, label it 100. Continue to the end of the transect (flag 900). In theory, the transect ends and plant plots are marked with nails.

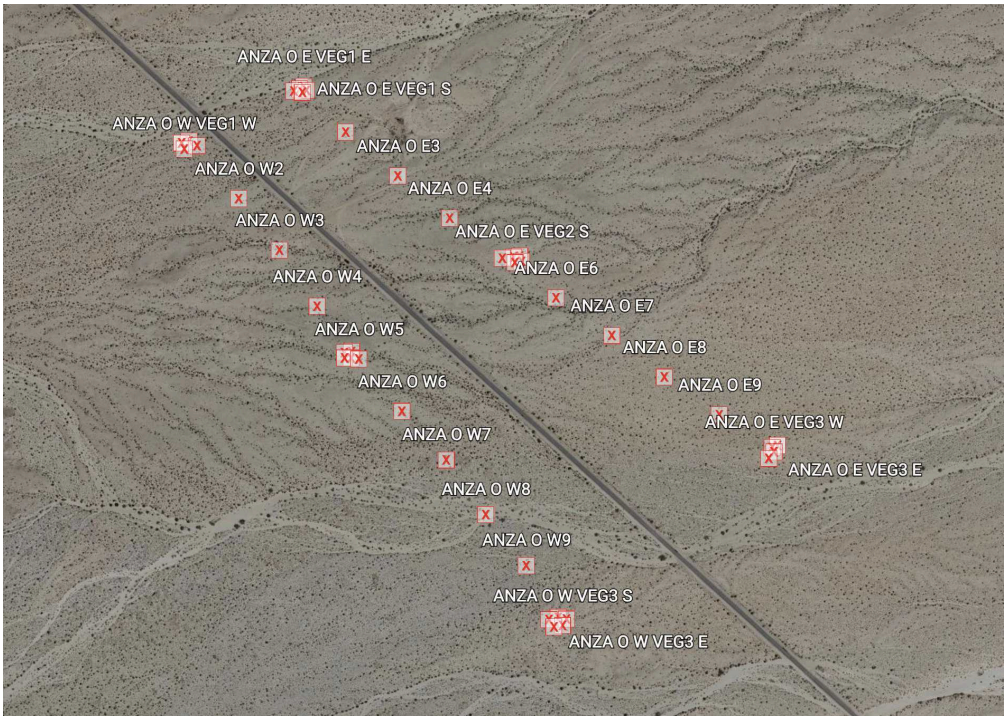
Veg plots: Each transect has three 10m² vegetation plots set ~10 m off at the beginning, middle and end of each transect. At some sites this placement of vegetation plots was not possible due to terrain so site maps/GPS points should be referenced. Use the GPS to find the approximate placement and mark the corners with pin flags.

These are the only acceptable site abbreviations. Please use them on all specimens and datasheets.

	A	B	C	D
	Site	DescriptiveSite	County	Veg Community

	A	B	C	D
	Anza O	Ocotillo/Borrega (along SR 2 north of Ocotilla)	Imperial	Desert scrub
	Anza S	S Anza Borrega State Park	Imperial	Desert scrub
	Echo O	Echo Valley/Descanso	San Diego	Chaparral
	JV O	Japutal Valley	San Diego	Burned chaparral
	Mt. Laguna O	Mt. Laguna	San Diego	Woodland
	TP O	Torrey Pines State Park	San Diego	Coastal Sage Scrub/Coastal Dunes

Southern transect sites.



Anza O site (BLM land) as an example. Two transects, netting and trapping are alternated between visits. Veg plots are located at each end (flag 1 and 10) and middle (flag 5) of each transect.

Anza veg plot example (at flag 10)

Pan/vane trap Setup

4 **Supplies:**

- **Trap weather datasheet**
- 5 vane traps
- 30 pan traps sets (10 each of blue, yellow, white)
- 3X 1L bottle of water
- 1mL dish soap
- Kestral
- pen/pencil/sharpie
- basket/trash bag for carrying traps

Note: you can only trap when the temperature is between 17-32C (60-90F) and the windspeed is below 2.5 m/s (only slightly colder than the net cut-off).
- You should not set up traps outside these conditions. Just chill in the car and enjoy the scenery if it is too cold or windy to sample in the early morning. If the weather gets bad throughout the day before 4 hours has passed, record how long the pans were out on the weather sheet, collect them, and finish the remainder the next day.

Set up:

1. Add 1mL of dish soap (can be prepared in eppendorf vials already) into 1L of water. Shake vigorously to mix the soap and water in the 1L bottle. Repeat with the other two bottles.
2. Record the weather on the trap weather datasheet
3. Navigate to the beginning of the transect and place either a vane trap or group of three pan traps (one of each color) ~1m from the nail and flagging tape or wherever there is flat ground.
4. Fill vane traps with ~5cm of soapy water (about the height of 3 fingers). Fill pan traps halfway with soapy water and space ~10cm apart from each other.
5. Place a vane or group of pan traps every 100 m along the transect, alternating between trap type (ex. if you place a vane trap at the first point, put pan traps at the second, and then a vane trap at the third, etc.) Pan traps are placed on the ground in a cluster, about 6-12 inches apart. Vane traps should be hung at vegetation height or placed on the ground
6. Leave the traps out for **4 hours**. During that time you should net and also do vegetation sampling if there is time.

Insect Collection

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Assemble your supplies in from your supply box:

- **Net weather datasheet**
- fieldnotes booklets
- net
- stopwatch
- non-sterile vials
- insulated fanny pack
- ice pack
- butterfly box + butterfly envelopes
- Kestral
- flat tweezer and pointy tweezer
- 2x thin sharpie(s) and 2x micron(s)
- Plant guide
- Opt: bleach bottle

In the car

- dry ice in Yeti (in the car)

Before starting to sample:
0. Walk the transect together as a crew noting the different plants that are blooming and their six-letter codes. It may be helpful to write them down in your field booklet. For plants the crew is unsure of, fill out the unknown plant guide together and agree on a consistent code to use. This can be done while setting up the traps.

Note: you can only net when the temperature is between 18-32C (65-90F) and the windspeed is below 2.5 m/s.

1. Navigate to the beginning of the other transect(not the transect you put the traps on). Look for the flagging marking the next section of the transect or keep an eye on the GPS to make sure you are staying close to the transect while you net.
2. Check that your stopwatch is set to Countdown and shows **100 minutes** (a total of 100 minutes will be collectively sampled at each transect per round, if 2 people are sampling, timers can be set to 50 mins each).
3. Start your stopwatch and begin looking for bees. You will stop the stopwatch every time you catch a bee, and start it again after you have put the bee in a vial and are ready to begin searching for a new specimen (so you are not counting any time to get the bee out of the net, into a vial, or labeled in the sampling time). Start your stopwatch and slowly walk towards the second point on the transect so that it takes you **10 minutes (of active search time) to cover 100 m.** You should zigzag a bit so that you cover a few meters on either side of the transect and don't just walk in a perfectly straight line.
4. You should collect any pollinating insect that touches the reproductive parts of a flower within your assigned subsite. This can include bees, flies, butterflies, and wasps (sometimes beetles). Capture bees, flies etc into vials. For butterflies, use the envelopes located in the butterfly box.
5. If you get a particularly pollen-covered insect that smears pollen all over your net, spray the bag with bleach (destroy pollen), wait 30 seconds, then ethanol (helps evaporation). Wave it around to dry it.
6. You should **ignore any beetles that you know do not pollinate (just sitting in the flower), hemipterans, grasshoppers, ants, ladybugs, spiders, etc. Do not collect an insect if it is just sitting on the petals or leaves --only if it seems to be foraging and actively engaging in pollination.**

Once you have a specimen in your net, stop your stopwatch and transfer it into a vial, you should label this vial carefully so that it is legible using your sharpie with the:

1. **site code (ie Anza O)**
2. **transect ID (generally a cardinal direction, E, W etc.) and transect section (1-9)**
3. **your initials, date**
4. **the 6-letter code for the flower from which you collected the insect**

Make sure to leave enough space for the unique ID number. Please write very legibly on the vials and ensure that it does not rub off when handling. When we add the unique number label, use this time to double-check the information is still clearly written.

Honey bees: There are a lot of honey bees. Collect as much as is reasonable (not spending all your time collecting honey bees) and try to get a spectrum of different plants. If you collect 1 honey bee on plant X but there are 15 more, write + 14 HB on that honey bee's vial. Then transcribe those counts to the honey bee data sheet.

Unsure of a plant ID? If you are not sure about the flower, create a temporary plant code based on the information you know such as color and family (small, white, 5-petals, opposite leaves), then you must write a description of the plant in as much detail as possible, and take multiple photos. Use the **Unknown plant datasheet** to write the description. **Upload the photos to the iNaturalist** and to **Dropbox** labeled with the code you used on vials and the site (i.e., thistle_white_AnzaO)**.** Update the unknown plant datasheet you have an ID from iNaturalist.

Store the collected insect in your fanny pack on an ice pack, to keep the insect cool and calm.

A note about flower labeling:
For this project, we use a labeling system for flowers in which we only write the first three letters of the genus and species (so Hymenoxys hoopesii becomes "HYMHOO" or Astragalus trichopodus var. lonchus becomes "ASTTRI"). Capitalization and spacing does not matter, as long as you can tell what the 6-letter code is when you copy head labels back in the lab.

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Once you have completed your sampling for the day:

Supplies

- **Specimen datasheet**
- **Honey bee datasheet**
- **Unknown flower datasheet**
- pre-printed numbered labels
- freezer box
- freezer ziplock
- sharpie

1. Put the vials in the vial box. For data entry try to keep different collector's specimens grouped together. Use this time to check over the data on the vials. If some flowers are still un-IDed, take some time to try to ID them and correct the plant code. If you cannot ID the plant, fill out the Unknown flower datasheet.
2. Count the vials. Place a cap sticker on the first vial and last vial. Referring to the unique IDs used in the last round, write the first and last number on the cap. You can alternate cap sticker colors between days/sites. Record which label numbers were used on the specimen datasheets.
3. Put all the vials from that collection day into a freezer box label the box with: **Site, date of collection, number range from that collection period. EX ECHO O, 03/16/2022 #4567-4800**
4. Transcribe any additional honey bee counts into the **honey bee datasheet**

5. Place the freezer box into a freezer ziplock bag labeled with the site and date
6. Put that bag into the dry ice cooler to kill the insects, put it in the freezer when you get to the UC field station.

Note: once the vials are placed in the cooler with dry ice you cannot write on the vials due to condensation.

Pan Trap Collection

7 Supplies:

- **Trap Weather datasheet**
- 18-20 non-sterile vials filled with 70% ethanol, or a squirt bottle of 70% ethanol
- pan trap label(s)
- micron pen
- scissors
- flat forceps
- fine tip forceps
- kestral

Traps should be out for as close to **4 hours** as possible. Do not pick the traps up earlier than 4 hours, unless it rains and they need to be collected early.

Note: if the weather becomes inappropriate for sampling (i.e. temperature drops below 17C, windspeed goes over 2.5m/s, it becomes too overcast to cast a shadow), make a note of the times that it is inadequate sampling weather on the Pan Weather sheet. Any time that is not appropriate for sampling does not count toward the 4 hours of pan trap time (so for instance, if you put out the pan traps at 10:00am, and at 12:30, it becomes too overcast until 1:00pm, you need to add an extra half hour to the pan trap time, so instead of picking them up at 2:30, you will pick them up at 3:00). If it is raining, or weather is consistently poor, then you should record the weather, stop the time, and retrieve the traps/process specimens as described below. New weather will need to be taken when they are set out again to complete their 4 hour time.

1. Once you have determined that it is time to collect traps, take the weather and note it on the pan weather sheet.
2. Fill out the trap labels **using a micron pen** and take a bag of non-sterile vials filled with ethanol.
3. Collect all the flower-visiting **insects** in each pan trap and put in one **70% ethanol vial** with an appropriate label in the vial (so if you are collecting the blue pan at the south flag, put the label that says S B in the vial). You can use the flat tweezers or the strainer, or a combination of both.
4. Remove insects from vane or pan traps by pouring the water through the strainer or using the flat/fine forceps to pick out the insects and place in a tube with ethanol and a label. *if all insects do not fit, make an additional vial.

5. If there are no specimens in a trap, simply dump out the water, no notation is necessary.
6. Once all insects and traps have been picked up, finish filling out the weather datasheet that was started when the traps were set out.
7. Put all the vials in a labeled ziplock (site, date) and put the non-dry ice cooler (so keep the specimens cold not they do not need to be frozen at low temperatures). You do not need to put ID number stickers on these vials because they will be pinned individually.

Note: If there are too many insects to fit into a single vial, you can use a second vial, but make sure to copy all of the relevant information onto a second label (or blank piece of label paper). Do not just write the information on the vial, since this will get rubbed off because of the ethanol.

Note: Only insects that would act as flying pollinators need to be collected from the traps (**you do not need to collect ants, grasshoppers, hemipterans, ladybugs, spiders, any insect/creature that is obviously not a pollinator). PLEASE discard any non-pollinators because it makes a lot of work down the line).**

Vegetation Quadrats

8 Supplies:

- **Plant survey datasheet**
- pen/pencil
- plant guides

Vegetation surveys are not timed or weather-sensitive and can be before, in between, or after pollinator surveys.
Each site has a total of six vegetation plots (6 one each transect) but only the three will be sampled during a site visit. Vegetation surveys are always done on the same transect that is actively netted.

1. Navigate to each corner of **10m² plot** to get a sense of the entire plot. (Try not to walk through the plot as much as possible to reduce trampling the vegetation being surveyed!)
2. For each plot fill out a data sheet with site name, date, and initials of all individuals collecting the data
3. For the initial site visit all plants present in the plot will be recorded; trees, shrubs, herbaceous perennials and annuals, and grasses. A plant must be rooted inside the plot to count.
4. For each subsequent site visit, only flowering plants will be recorded.

5. Plants are recorded by using the first three letters of the genus and the first three letters of the species. Ex: *Acmispon glaber* would become ACMGLA (just as in the net sureveys).
6. If an unknown plant is encountered, write a short description in the **unknown plant datasheet** and take photos to show the overall growth form of the plant, the leaf shape and arrangement, and the flower if one is present. Using a hand or clipboard in photos to show size/scale can be extremely helpful for identifying the plant. Note on the datasheet who has the photos. Upload them to iNaturalist and dropbox. Update the unknown data sheet with the final ID.
7. For each plant recorded, you will also record bloom description, count of individuals, and number of blooms.

- While not all individuals in the plot may not be at the exact same stage of blooming, record what characterizes the majority of individuals.
- Counts of individuals should be as specific as possible but can be rounded with large numbers of individuals. Ex: 20-100 can be rounded to the nearest ten, 100-200 rounded to the hundred, over 1000 to the nearest thousand.
- **Tightly packed inflorescences should be counted as a single bloom. Ex: *Cryptantha* has a cyme with multiple flowers but each cyme should be counted as a single bloom. Sames goes for the inflorescences of *Ceanothus*, *Eriogonumfasciculari*s, and *Ambrosia dumosa.***
- If multiple data sheets are used, write “1 of 2” and “2 of 2.”

End of Day

9

Supplies:

- **Laptop**
- smartphone

The team leader is responsible for ensuring that all data is entered on the laptop in the following folder:
Dropbox → ca_resurvey_fieldwork_2022 → raw_data

The team leader does not need to enter all the data by themselves, but check that it's been done and do random spot checks in each datasheet to ensure the quality of data entry.

After data entry, each datasheet should be scanned with the image scanner and uploaded onto Dropbox:
Dropbox → ca_resurvey_fieldwork_2022 → raw_data → scanned datasheets

Field station storage and pinning

10 **Supplies:**

- Laptop
- Barcoded labels
- Pinning kit
- Netted specimen boxes
- Trap specimens in ethanol in labeled ziplocks

Storage:

- Store all netted and pan specimens in the lab freezer (-20). Record the freezer location in the specimen datasheet. Keep specimens together to avoid them getting lost.

Data:

- The crew leader is responsible for ensuring that all data is entered on the laptop in the following folder: Dropbox → ca_resurvey_fieldwork_2022 → raw_data
- Save often, ensure dropbox is syncing
- The team leader does not need to enter all the data by themselves, but check that it's been done and do random spot checks in each datasheet to ensure the quality of data entry.

Pinning:

- Each specimen will get a barcoded label. Follow the pinning protocol (pdf in dropbox) and add a barcoded label to each specimen and pin them in order into the drawers. Enter the specimen's data in dropbox as you go. Add the unique ID assigned to the specimen to the tube (sharpie) and put a star on the lid if there is pollen. Replace the vial in the vial box in order.
- Try to only pin if you can finish an entire box to avoid freezing and dethawing
- Store the box empty in the freezer with the other specimens to avoid them getting lost (if there's space in the freezer) otherwise keep them together in a cabinet. The boxes come back to UO (in case there are data issues that need resolution)