**## Supplemental File S7. Coding for all graphs and confidence interval statistics.**

**## Title of article:** Triplication: an important component of the modern scientific method.

**## Authors:** Jeremy S.C. Clark, Karina Szczypiór-Piasecka, Kamila Rydzewska, Konrad Podsiadło.

## Article figures are given with letters in the coding, e.g. FigureA (or 0A/0B).

## Instructions: (1) Remove the first two rows from the file S9\_Supplemental\_File\_S9\_Collated.xlsx and convert to .csv; (2) put this .csv file into a folder; find path to folder using R file.choose() and put as mypath below without the file name; unhash coding below to install R [educate]; copy all code into R and run.

mypath <- ""

options(timeout=1000)

options(java.parameters = "-Xmx8000m")

## install.packages("devtools")

## library("devtools")

## devtools::install\_github("zief0002/educate") ## turn off vpn

ipak <- function(pkg){

new.pkg <- pkg[!(pkg %in% installed.packages()[, "Package"])]

if (length(new.pkg))

install.packages(new.pkg, dependencies = TRUE, repos="http://cran.r-project.org")

sapply(pkg, require, character.only = TRUE)

} ## end function

packages <- c("effectsize", "rcompanion", "pwr", "purrr", "broom", "DescTools", "Hmisc", "XNomial", "rlist", "plyr", "cowplot", "ggtext", "ggarrow", "ggforce", "forcats", "dplyr", "data.table", "ggplot2", "ggpattern", "tidyverse")

ipak(packages) ## References given at end of document.

## cbp2 <- c("#D55E00", "#56B4E9", "#009E73", "#E69F00", "#F0E442", "#0072B2", "#CC79A7", "#000000") ## color-blind with black

cbp2 <- c("#D55E00", "#56B4E9", "#00d99e", "#009E73", "#0072B2", "#5D3A9B", "#CC79A7", "#000000") ## color-blind with black

## dbp2 <- c("solid", "twodash", "longdash", "dotdash", "dashed")

dbp2 <- c("solid", "91", "519191", "71", "5191", "13")

linetypes <- function(n) {

types <- c("55", "75", "95", "1115", "111115", "11111115", "5158", "9198", "c1c8")

return(types[seq\_len(n)])

}

dev.new()

scales::show\_col(cbp2)

##

scale\_colour\_discrete <- function(...) {

ggplot2::scale\_colour\_manual(values = cbp2, ...)

}

scale\_fill\_discrete <- function(...) {

ggplot2::scale\_fill\_manual(values = cbp2, ...)

}

theme\_jack <- function (base\_size = 16, font = "Helvetica", base\_family = "", axisColor='#999999', textColor='black') {

theme\_classic(base\_size = base\_size, base\_family = base\_family) %+replace%

theme(

plot.title = element\_text(size=15, vjust=3),

axis.text.x = element\_text(colour = "black", family="Times", face=c('bold'), size = 18, vjust = 0.2),

axis.text.y = element\_text(colour = "black", family="Times", face=c('bold'), size = 18),

axis.title.x = element\_text(colour = "black", family="Times", face=c('bold'), vjust = -4, size = 20),

axis.title.y = element\_text(colour = "black", family="Times", angle=90, face=c('bold'), vjust= 5, size = 20),

panel.grid.minor = element\_blank(),

panel.grid.major = element\_blank(),

plot.background = element\_rect(fill="white", color = "white"),

panel.border = element\_blank(),

panel.background = element\_blank(),

plot.margin=unit(c(1,1.5,1.3,1.3),"cm")

)

}

theme\_set(theme\_jack())

## THE FOLLOWING CHANGES ggplot function !! adds 10% to y-axis. To revert to standard use ggplot <- function(...) ggplot2::ggplot(...) or ggplot2::ggplot(content). MUST define xxx and yyy in loop before using ggplot

ggplotFB <- function(...) ggplot2:::ggplot(...) + {if (all(xxx >= 0, na.rm = TRUE)) scale\_x\_continuous(expand = c(0, 0), limits = c(0, NA))} + {if (all(yyy >= 0, na.rm = TRUE)) scale\_y\_continuous(expand = c(0, 0), limits = c(0, NA))} + {if ((all(xxx >= 0, na.rm = TRUE)) & (all(yyy >= 0, na.rm = TRUE))) coord\_cartesian(xlim = c(0, max(xxx, na.rm = TRUE)), ylim = c(0, max(yyy, na.rm = TRUE) + max(yyy, na.rm = TRUE)/10))}

integer\_breaks <- function(n = 5, ...) {

fxn <- function(x) {

breaks <- floor(pretty(x, n, ...))

names(breaks) <- attr(breaks, "labels")

breaks

}

return(fxn)

}

int\_breaks\_drop\_zero <- function(n = 5, ...) { ## use -breaks=int\_breaks\_drop\_zero(n = 7))

fxn <- function(x) {

breaks <- floor(pretty(x, n, min.n = 0, ...))

breaks <- breaks[breaks != 0]

if (length(breaks) == 0) return(NULL)

names(breaks) <- attr(pretty(x, n, min.n = 0, ...), "labels")

breaks

}

return(fxn)

}

**## Figures 0A and 0B. Variable construct.**

set.seed(567)

P <- c(13, rep(22, 3), rep(40, 4), rep(55.5, 2), 19.0, 39.5, 35.7, 60, 70)

x1 <- rep("P", length(P))

Q <- c(20, rep(40, 2), rep(50, 3), rep(60, 3), rnorm(5, 80, 15), rep(80, 4), rep(90, 3), 100)

x2 <- rep("Q", length(Q))

df1 <- data.frame(x1, P)

colnames(df1) <- c("x", "y")

df2 <- data.frame(x2, Q)

colnames(df2) <- c("x", "y")

dfType <- rbind(df1, df2)

dfType

plotType0Atmp <- ggplot(data = dfType, aes(x = x, y = y)) + geom\_boxplot() +

xlab("Categorically distinct groups") + ylab(str\_wrap("Continuous main effect parameter", width = 25)) + scale\_y\_continuous(breaks = c(0, 25, 50, 75, 100, 120), labels = c("", "", "", "", "", ""), expand = c(0, 0), limits = c(0, 120))

plotType0A <- cowplot::ggdraw(plotType0Atmp) + draw\_figure\_label(label = "a.", position = c("top.left"), size = 25, fontface = "bold")

dev.new()

plotType0A

ggsave("Figure0A\_Science\_type.pdf", plot = plotType0A, device = "pdf", width = 6, height = 5, units = "in", dpi = 300, path = mypath)

plotType0Btmp <- ggplot(data = dfType, aes(x = x, y = y)) + geom\_count(color = cbp2[[5]], na.rm = TRUE) + scale\_size\_area(max\_size = 15) +

xlab("Categorically distinct groups") + ylab(str\_wrap("Continuous (or ordinal) main-effect parameter", width = 25)) + scale\_y\_continuous(breaks = c(0, 25, 50, 75, 100, 120), labels = c("", "", "", "", "", ""), expand = c(0, 0), limits = c(0, 120)) +

theme(legend.position = "none")

plotType0B <- cowplot::ggdraw(plotType0Btmp) + draw\_figure\_label(label = "b.", position = c("top.left"), size = 25, fontface = "bold")

dev.new()

plotType0B

ggsave("Figure0B\_Science\_type.pdf", plot = plotType0B, device = "pdf", width = 6, height = 5, units = "in", dpi = 300, path = mypath)

**## Collated replication results:**

collated <- fread(paste0(mypath, "S9\_Supplemental\_File\_S9\_Collated.csv"), header = TRUE)

collated <- as.data.frame(collated)

collated <- collated[1:80, ]

conclusion <- collated$Conclusion

table (conclusion)

## > table (conclusion)

## conclusion

## Double result (repetition) protocol

## 11

## Global (duplicated-methods) protocol

## 4

## Global (replication-error) protocol (s.d.)

## 6

## Global (replication-error) protocol (s.e.)

## 3

## Global (replication-error) protocol (variance)

## 1

## Global (triplicated-methods) protocol

## 33

## Non-replicated protocol

## 10

## Triple result (repetition) protocol

## 12

##

nrow(collated)

collated$con2 <- collated$Conclusion

collated$con2[collated$con2 == "Global (replication-error) protocol (s.d.)"] <- "Global (replication-error) protocol"

collated$con2[collated$con2 == "Global (replication-error) protocol (s.e.)"] <- "Global (replication-error) protocol"

collated$con2[collated$con2 == "Global (replication-error) protocol (variance)"] <- "Global (replication-error) protocol"

df <- as.data.frame(table(collated$con2))

df

df$group <- c("D", "G2", "G3e", "G3p", "NoR", "T")

neworder <- c("NoR", "G2", "D", "G3p", "G3e", "T")

df <- df %>% slice(match(neworder, group))

colnames(df) <- c("Var1", "Frequency", "group")

df$Proportion <- df$Frequency /80

df$Percentage <- paste0(round(df$Proportion \* 100, digits = 0), "%")

df$Percentage <- factor(df$Percentage, levels = unique(df$Percentage))

df$group <- factor(df$group, levels = c("NoR", "G2", "D", "G3e", "G3p", "T")) ## prevents alphabetical ordering by ggplot !

df$Protocol <- c("NoR", "G2", "D", "G3", "G3", "T")

df$Protocol <- factor(df$Protocol, levels = unique(df$Protocol))

## df$position <- c(1, 1, 2, 1, 2, 1)

## df$position <- factor(df$position, levels=c(1, 1, 2, 1, 2, 1))

## > df

## Var1 Frequency group Proportion Percentage Protocol

## 1 Non-replicated protocol 10 NoR 0.1250 12% NoR

## 2 Global (duplicated-methods) protocol 4 G2 0.0500 5% G2

## 3 Double result (repetition) protocol 11 D 0.1375 14% D

## 4 Global (triplicated-methods) protocol 33 G3p 0.4125 41% G3

## 5 Global (replication-error) protocol 10 G3e 0.1250 12% G3

## 6 Triple result (repetition) protocol 12 T 0.1500 15% T

cbp2B <- c("#56B4E9", "#56B4E9", "#56B4E9", "#56B4E9", "#0072B2", "#56B4E9")

names(cbp2B) <- df$group # Named vector for proper mapping

myPattern <- c("none", "none", "stripe", "none", "stripe", "stripe")

names(myPattern) <- df$group

myAngle <- c(45, 135, 45, 135, 0, 45)

names(myAngle) <- df$group

legendPattern <- c("none", "none", "stripe", "none", "stripe", "stripe")

names(legendPattern) <- df$group

Patterncolor <- c("#56B4E9", "#56B4E9", "#56B4E9", "#56B4E9", "#0072B2", "#56B4E9")

names(Patterncolor) <- df$group

myVjust <- c(4, 2, 4.5, 6, 4, 5.2)

legend\_groups <- c("G3p", "D", "G3e")

myLabels <- c("NoR or Global", "Result reps", "G3e")

plotProtocols <- ggplot(data = df, aes(x = Protocol, y = Proportion, fill = group, pattern = group)) + geom\_bar\_pattern(

stat = "identity", position = "stack", color = "black",

pattern\_fill = "black", pattern\_color = Patterncolor,

pattern\_angle = myAngle, pattern = myPattern

) +

scale\_fill\_manual(values = cbp2B, breaks = legend\_groups, labels = myLabels) +

scale\_pattern\_manual(values = myPattern, breaks = legend\_groups) +

guides(fill = guide\_legend(override.aes = list(pattern = legendPattern[legend\_groups], pattern\_angle = myAngle[legend\_groups], pattern\_color = c("#56B4E9", "#56B4E9", "#0072B2"))), pattern = guide\_legend(override.aes = list(pattern = legendPattern[legend\_groups], pattern\_angle = myAngle[legend\_groups], pattern\_color = "#0072B2"))) + labs(fill = "") + xlab("Protocol") + ylab("Prop. of studies (%)") +

scale\_y\_continuous(breaks = c(0, 0.2, 0.4, 0.6), labels = c("0", "20", "40", "60"), expand = c(0, 0), limits = c(0, 0.6)) + geom\_text(aes(label = Percentage), position = position\_stack(), vjust =myVjust, color="white", size=3.5) + theme(legend.position = "inside", legend.position.inside = c(0.25, 0.8))

## graphics.off()

## dev.off()

## dev.new(width=7, height=5)

## grid::grid.newpage(recording = TRUE)

plotProtocols

ggsave("FigureAA\_protocol\_props.pdf", plot = plotProtocols, device = "pdf", width = 6, height = 5, units = "in", dpi = 300, path = mypath)

collated$Num\_reps <- ifelse((grepl("Yes", collated$More\_or\_not)) & (!grepl("4", collated$More\_or\_not)), "Yes", ifelse(grepl("4", collated$More\_or\_not), "4", ifelse(grepl("no", collated$More\_or\_not), "no", NA)))

collated$exact <- collated$con2

collated$exact[(collated$Num\_reps == "4") & (collated$con2 == "Global (triplicated-methods) protocol")] <- "G4"

collated$exact[(collated$Num\_reps == "4") & (collated$con2 == "Triple result (repetition) protocol")] <- "T4"

collated$exact[(collated$Num\_reps == "Yes") & (collated$con2 == "Global (triplicated-methods) protocol")] <- "G5+"

collated$exact[(collated$Num\_reps == "Yes") & (collated$con2 == "Triple result (repetition) protocol")] <- "T5+"

collated$exact

collated$exactcode <- collated$exact

collated$exactcode[collated$exactcode == "Triple result (repetition) protocol"] <- "T"

collated$exactcode[collated$exactcode == "Double result (repetition) protocol"] <- "D"

collated$exactcode[collated$exactcode == "Global (replication-error) protocol"] <- "G3e"

collated$exactcode[collated$exactcode == "Global (triplicated-methods) protocol"] <- "G3p"

collated$exactcode[collated$exactcode == "Non-replicated protocol"] <- "NoR"

collated$exactcode[collated$exactcode == "Global (duplicated-methods) protocol"] <- "G2"

collated$exactcode <- factor(collated$exactcode, levels = c("NoR", "G2", "D", "G3e", "G3p", "T", "G4", "T4", "G5+", "T5+")) ## prevents alphabetical ordering by ggplot !

mygroup <- c("NoR", "G2", "D", "G3", "T", "G4", "T4", "G5+", "T5+")

dfexact <- as.data.frame(table(collated$exactcode))

dfexact

colnames(dfexact) <- c("Protocol", "Frequency")

dfexact$Proportion <- dfexact$Frequency /80

dfexact$Percentage <- paste0(round(dfexact$Proportion \* 100, digits = 0), "%")

dfexact$Percentage <- factor(dfexact$Percentage, levels = unique(dfexact$Percentage))

dfexact$group <- as.character(dfexact$Protocol)

dfexact$group[dfexact$group == "G3e"] <- "G3"

dfexact$group[dfexact$group == "G3p"] <- "G3"

dfexact$group <- factor(dfexact$group, levels = unique(dfexact$group))

**## Attempt at estimation of exponential parameters: for counts, variance will increase with mean - so use multiplicative errors:**

## ## errors grow as y grows - multiplicative (constant on the log-scale), e.g., simulation:

## ## y\_mult <- a\*exp(r\*t + rnorm(length(t), sd = 1)) # or: rlnorm(length(t), mean = log(a) + r\*t, sd = 1). Estimation:

## ## mult\_lm <- lm(log(y\_mult) ~ t)

## ## coef(mult\_lm)

##

##

## names <- c("non", "dup", "trip", "quad")

## numbers <- c(1, 2, 3, 4)

## values <- c(10, 15, 40, 7)

## combineddf <- data.frame(names, numbers, values)

## combineddf

## mult\_lm <- lm(log(values) ~ numbers)

## mult\_lm

## ## Coefficients:

## ## (Intercept) numbers

## ## 2.68366 -0.00892

## Intercept <- exp(2.68366)

## Intercept

## ## 14.63857

## ## y = a \* er\*t

## yy <- Intercept \* exp(-0.00892\*numbers)

## yy

## ## 14.50858 14.37974 14.25204 14.12548 ## very similar to mean - not used.

**## Preparation for test for differences between the proportions: (G3e and G3p combined)**

dfmultiB <- dfexact[!(dfexact$Protocol %in% "G3e") & !(dfexact$Protocol %in% "G3p"), ]

G3df <- data.frame(Protocol = "G3", Frequency = 33, Proportion = 0.1250 + 0.2875, Percentage = "41%", group = "G3")

dfmulti <- rbind(dfmultiB[1:3, ], G3df, dfmultiB[4:8, ])

dfmulti$equal <- rep(80 \* 1/nrow(dfmulti), nrow(dfmulti))

rownames(dfmulti) <- NULL

dfmulti

dfmulti$equalprop <- dfmulti$equal / 80

dfmulti$equalprop

## dfmulti

## Protocol Frequency Proportion Percentage group equal equalprop

## 1 NoR 10 0.1250 12% NoR 8.888889 0.1111111

## 2 G2 4 0.0500 5% G2 8.888889 0.1111111

## 3 D 11 0.1375 14% D 8.888889 0.1111111

## 4 G3 33 0.4125 41% G3 8.888889 0.1111111

## 5 T 7 0.0875 9% T 8.888889 0.1111111

## 6 G4 3 0.0375 4% G4 8.888889 0.1111111

## 7 T4 4 0.0500 5% T4 8.888889 0.1111111

## 8 G5+ 7 0.0875 9% G5+ 8.888889 0.1111111

## 9 T5+ 1 0.0125 1% T5+ 8.888889 0.1111111

**## Power studies for prediction of sample sizes needed for the multinomial are given in Supplemental\_File\_S11.**

**## Multinomial exact Monte-Carlo p-values: small-cell adjustment not needed because of (1x107) Monte Carlo trials:**

## XNomial::xmonte(dfmulti$Frequency, dfmulti$equal, ntrials = 1e+07, statName = "Prob", histobins = F, histobounds = c(0, 0), showCurve = FALSE, detail = 2, safety = 1e+12)

## P value (Prob) = 0 ± 0 ## taken to be p<1e-07

## 1e+07 random trials

## Observed: 10 4 11 33 7 3 4 7 1

## Expected Ratio: 8.888889 8.888889 8.888889 8.888889 8.888889 8.888889 8.888889 8.888889 8.888889

**## Evaluation of actual effect size for protocol proportion result:**

tbl <- matrix(dfmulti$Frequency, nrow = 1)

tbl

cw\_effectsize <- effectsize::cohens\_w(tbl)

cw\_effectsize

## Cohen's w | 95% CI

## ------------------------

## 1.02 | [0.79, 2.83]

##

## - One-sided CIs: upper bound fixed at [2.83~].

**## post-hoc binomial tests (from https://rcompanion.org/ Salvatore Mangnifico), and Wilson's confidence intervals (these behave well with small cell sizes):**

dfbin <- data.frame(dfmulti$Frequency, rep(80, nrow(dfmulti)), rep(9, nrow(dfmulti)), rep(80, nrow(dfmulti)))

colnames(dfbin) <- c("Successes", "Total", "Numerator", "Denominator")

dfbin[4, "Numerator"] <- 8

dfbin

funp <- function (x){

stats::binom.test(x["Successes"], x["Total"], x["Numerator"]/x["Denominator"])$p.value

}

funest <- function (x){

DescTools::BinomCI(x["Successes"], x["Total"], conf.level = 0.95, sides = c("two.sided"), method = c("wilson"))[[1]]

}

funlwr <- function (x){

signif(DescTools::BinomCI(x["Successes"], x["Total"], conf.level = 0.95, sides = c("two.sided"), method = c("wilson"))[[2]], 4)

}

funupr <- function (x){

signif(DescTools::BinomCI(x["Successes"], x["Total"], conf.level = 0.95, sides = c("two.sided"), method = c("wilson"))[[3]], 4)

}

dfbin$p.value <- apply(dfbin, 1, funp)

dfbin$p.value <- signif(dfbin$p.value, 3)

dfbin$p.bonf <- dfbin$p.value \* 9 ## Bonferroni correction

dfbin$est <- apply(dfbin, 1, funest)

dfbin$lwr <- apply(dfbin, 1, funlwr)

dfbin$upr <- apply(dfbin, 1, funupr)

dfbin$group <- mygroup

dfbin

##Successes Total Numerator Denominator p.value p.bonf est lwr upr group

## 1 10 80 9 80 7.22e-01 6.498e+00 0.1250 0.069335576 0.2150280 NoR

## 2 4 80 9 80 7.77e-02 6.993e-01 0.0500 0.019613555 0.1216228 G2

## 3 11 80 9 80 4.77e-01 4.293e+00 0.1375 0.078547143 0.2296710 D

## 4 \*\*\* 33 80 8 80 2.66e-13 2.394e-12 0.4125 0.311058576 0.5219596 G3

## 5 7 80 9 80 5.96e-01 5.364e+00 0.0875 0.043032002 0.1697680 T

## 6 3 80 9 80 3.20e-02 2.880e-01 0.0375 0.012834569 0.1045472 G4

## 7 4 80 9 80 7.77e-02 6.993e-01 0.0500 0.019613555 0.1216228 T4

## 8 7 80 9 80 5.96e-01 5.364e+00 0.0875 0.043032002 0.1697680 G5+

## 9 \* 1 80 9 80 1.24e-03 1.116e-02 0.0125 0.002209976 0.0674627 T5+

## p \* <0.05, \*\* < 0.01, \*\*\* < 0.001

meanest <- mean(dfbin$est)

meanest

## 0.1111111

**## Effect sizes:**

## Salvatore(2025), Cohen(2013):

myCohenH <- 2 \* asin(sqrt(dfbin$est)) - 2 \* asin(sqrt(8.89/80))

myCohenH

dfbin$CohenHest <- signif(myCohenH, 4)

**## confidence intervals for Effect size Cohen's H taken from Wilson CI:**

lwrCI\_H <- 2 \* asin(sqrt(dfbin$lwr)) - 2 \* asin(sqrt(8.89/80))

lwrCI\_H

dfbin$lwrCI\_H <- signif(lwrCI\_H, 4)

uprCI\_H <- 2 \* asin(sqrt(dfbin$upr)) - 2 \* asin(sqrt(8.89/80))

uprCI\_H

dfbin$uprCI\_H <- signif(uprCI\_H, 4)

## Successes Total Numerator Denominator p.value p.bonf est lwr upr group CohenHest lwrCI\_H uprCI\_H

## 1 10 80 9 80 7.22e-01 6.498e+00 0.1250 0.06934 0.21500 NoR 0.04302 -0.1468 0.28460

## 2 4 80 9 80 7.77e-02 6.993e-01 0.0500 0.01961 0.12160 G2 -0.22870 -0.3987 0.03267

## 3 11 80 9 80 4.77e-01 4.293e+00 0.1375 0.07855 0.22970 D 0.08004 -0.1116 0.31990

## 4 33 80 8 80 2.66e-13 2.394e-12 0.4125 0.31110 0.52200 G3 0.71520 0.5037 0.93510

## 5 7 80 9 80 5.96e-01 5.364e+00 0.0875 0.04303 0.16980 T -0.07912 -0.2618 0.16970

## 6 3 80 9 80 3.20e-02 2.880e-01 0.0375 0.01283 0.10450 G4 -0.29000 -0.4527 -0.02136

## 7 4 80 9 80 7.77e-02 6.993e-01 0.0500 0.01961 0.12160 T4 -0.22870 -0.3987 0.03267

## 8 7 80 9 80 5.96e-01 5.364e+00 0.0875 0.04303 0.16980 G5+ -0.07912 -0.2618 0.16970

## 9 1 80 9 80 1.24e-03 1.116e-02 0.0125 0.00221 0.06746 T5+ -0.45560 -0.5857 -0.15420

cbp2B <- c("#56B4E9", "#56B4E9", "#56B4E9", "#0072B2", "#56B4E9", "#56B4E9", "#56B4E9", "#56B4E9", "#56B4E9", "#56B4E9")

names(cbp2B) <- dfexact$Protocol

myPattern <- c("none", "none", "stripe", "stripe", "none", "stripe", "none", "stripe", "none", "stripe")

names(myPattern) <- dfexact$Protocol

myAngle <- c(45, 135, 45, 0, 135, 45, 90, 45, 90, 45)

names(myAngle) <- dfexact$Protocol

legendPattern <- c("none", "none", "stripe", "stripe", "none", "stripe", "none", "stripe", "none", "stripe")

names(legendPattern) <- dfexact$Protocol

myVjust <- c(4, 2, 4.5, 6, 6, 3, 2, 2, 2, 1)

names(myVjust) <- dfexact$Protocol

legend\_groups <- c("G3p", "D", "G3e")

myLabels <- c("NoR or Global", "Result reps", "G3e")

annotation1 <- data.frame(label = c("\*\*\*", "\*"), x = c(4.3, 9.2), y = c(0.45, 0.03))

plotProtocolexact <- ggplot(data = dfexact, aes(x = group, y = Proportion, fill = Protocol, pattern = Protocol)) +

geom\_bar\_pattern(stat = "identity", position = "stack", color = "black", pattern\_fill = "black", pattern\_color = "#0072B2", pattern\_angle = myAngle, pattern = myPattern) + scale\_fill\_manual(values = cbp2B, breaks = legend\_groups, labels = myLabels) + scale\_pattern\_manual(values = myPattern, breaks = legend\_groups) + guides(

fill = guide\_legend(override.aes = list(pattern = legendPattern[legend\_groups],

pattern\_angle = myAngle[legend\_groups],

pattern\_color = "#0072B2")),

pattern = guide\_legend(override.aes = list(pattern = legendPattern[legend\_groups],

pattern\_angle = myAngle[legend\_groups],

pattern\_color = "#0072B2"))) + labs(fill = "") + xlab("Protocol") + geom\_text(aes(label = Percentage), position = position\_stack(), vjust =myVjust, color="white", size=3.5) + theme(legend.position = "inside", legend.position.inside = c(0.86, 0.85)) + xlab("Protocol") + ylab("Prop. of studies (%)") + scale\_y\_continuous(breaks = c(0, 0.1, 0.2, 0.3, 0.4, 0.5), labels = c("0", "10", "20", "30", "40", "50"), expand = c(0, 0), limits = c(0, 0.55)) +

geom\_errorbar(data = dfbin, aes(x = group, ymin = lwr, ymax = upr), inherit.aes = FALSE, width = 0.4, color = cbp2[[1]], alpha = 0.9, linewidth = 1.3) + geom\_text(aes(label = Percentage), position = position\_stack(), vjust =myVjust, color="white", size=3.5) + theme(legend.position = "inside", legend.position.inside = c(0.86, 0.85)) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), inherit.aes = FALSE, color = cbp2[8], size=5, fontface="bold")

## graphics.off()

dev.new(width=7, height=5)

## grid::grid.newpage(recording = TRUE)

plotProtocolexact

ggsave("FigureAB\_protocol\_props.pdf", plot = plotProtocolexact, device = "pdf", width = 7, height = 5, units = "in", dpi = 300, path = mypath)

**## Associations within each group - exact**

dfassoc <- collated %>%

group\_by(collated$exact, collated$ASSOC\_EXP) %>% count()

dfassoc <- as.data.frame(dfassoc)

colnames(dfassoc) <- c("exact", "ASSOC\_EXP", "n")

dfassoc

## exact ASSOC\_EXP n

## 1 Double result (repetition) protocol Association 1

## 2 Double result (repetition) protocol Experimental 10

## 3 G4 Experimental 3

## 4 G5+ Association 1

## 5 G5+ Experimental 6

## 6 Global (duplicated-methods) protocol Experimental 4

## 7 Global (replication-error) protocol Association 2

## 8 Global (replication-error) protocol Experimental 8

## 9 Global (triplicated-methods) protocol Association 3

## 10 Global (triplicated-methods) protocol Experimental 20

## 11 Non-replicated protocol Association 2

## 12 Non-replicated protocol Experimental 8

## 13 T4 Experimental 4

## 14 T5+ Association 1

## 15 Triple result (repetition) protocol Association 1

## 16 Triple result (repetition) protocol Experimental 6

dfassoc$group <- dfassoc$exact

dfassoc$group[dfassoc$group == "Double result (repetition) protocol"] <- "D"

dfassoc$group[dfassoc$group == "Global (duplicated-methods) protocol"] <- "G2"

dfassoc$group[dfassoc$group == "Global (replication-error) protocol"] <- "G3e"

dfassoc$group[dfassoc$group == "Global (triplicated-methods) protocol"] <- "G3p"

dfassoc$group[dfassoc$group == "Non-replicated protocol"] <- "NoR"

dfassoc$group[dfassoc$group == "Triple result (repetition) protocol"] <- "T"

dfassoc$group[dfassoc$group == "G4"] <- "G4+"

dfassoc$group[dfassoc$group == "G5+"] <- "G4+"

dfassoc$group[dfassoc$group == "T4"] <- "T4+"

dfassoc$group[dfassoc$group == "T5+"] <- "T4+"

dfassoc$group <- factor(dfassoc$group, levels = c("NoR","G2", "D", "G3e", "G3p", "T", "G4+", "T4+"))

dfassoc

## exact ASSOC\_EXP n group

## 1 Double result (repetition) protocol Association 1 D

## 2 Double result (repetition) protocol Experimental 10 D

## 3 G4 Experimental 3 G4+

## 4 G5+ Association 1 G4+

## 5 G5+ Experimental 6 G4+

## 6 Global (duplicated-methods) protocol Experimental 4 G2

## 7 Global (replication-error) protocol Association 2 G3e

## 8 Global (replication-error) protocol Experimental 8 G3e

## 9 Global (triplicated-methods) protocol Association 3 G3p

## 10 Global (triplicated-methods) protocol Experimental 20 G3p

## 11 Non-replicated protocol Association 2 NoR

## 12 Non-replicated protocol Experimental 8 NoR

## 13 T4 Experimental 4 T4+

## 14 T5+ Association 1 T4+

## 15 Triple result (repetition) protocol Association 1 T

## 16 Triple result (repetition) protocol Experimental 6 T

## remove row 3:

dfassoc <- dfassoc[-3, ]

## change n for new row 4 to 9:

dfassoc[4, "n"] <- 9

dfassoc

tab <- dfassoc %>% group\_by(ASSOC\_EXP, group) %>%

summarise(n = sum(n), .groups = "drop") %>%

pivot\_wider(names\_from = group, values\_from = n, values\_fill = 0) %>% column\_to\_rownames("ASSOC\_EXP") %>% as.matrix()

print(tab)

fisher.res <- fisher.test(tab)

## Fisher's Exact Test for Count Data

##

## data: tab

## p-value = 0.9852

## alternative hypothesis: two.sided

##

**## Effect size for association result (confidence interval not given for this negative result):**

w <- rcompanion::cohenW(tab)

w

## Cohen w

## 0.1497

**## post-hoc power study for association result:**

N\_obs <- sum(tab)

pwr::pwr.chisq.test(w = w, df = 7, sig.level = 0.05, N = N\_obs) ## power with 80 articles

## Required N:

ceiling(pwr.chisq.test(w = w, df = 7, sig.level = 0.05, power = 0.80)$N)

## 641

**## Association / experimental graph**

cbp2 <- c("#D55E00", "#56B4E9", "#00d99e", "#009E73", "#0072B2", "#5D3A9B", "#CC79A7", "#000000") ## color-blind with black

dfassoc$myPattern[dfassoc$ASSOC\_EXP == "Association"] <- "stripe"

dfassoc$myPattern[dfassoc$ASSOC\_EXP == "Experimental"] <- "none"

dfassoc

myPattern <- dfassoc$myPattern

names(myPattern) <- dfassoc$group

myAngle <- rep(45, 15)

names(myAngle) <- dfassoc$group

## dev.new(width=10, height=5)

## grid::grid.newpage(recording = TRUE)

plotProtocolassoc <- ggplot(data = dfassoc, aes(x = group, y = n, fill = ASSOC\_EXP, pattern = ASSOC\_EXP)) + geom\_bar\_pattern(stat = "identity", position = "stack", color = "black", pattern\_color = cbp2[1], pattern\_fill = "black", pattern\_angle = myAngle, pattern = myPattern) +

guides(fill = guide\_legend(override.aes = list(pattern = myPattern[1:2], pattern\_angle = myAngle[1:2], pattern\_color = cbp2[1:2], pattern\_key\_scale\_factor = 0.8))) + labs(fill = "") + xlab("Protocol") + ylab("Number of studies") +

scale\_y\_continuous(breaks = c(0, 5, 10, 15, 20, 25), labels = c("0", "5", "10", "15", "20", "25"), expand = c(0, 0), limits = c(0, 25)) + theme(legend.position = "inside", legend.position.inside = c(0.87, 0.85))

dev.new()

plotProtocolassoc

ggsave("FigureB\_assoc.pdf", plot = plotProtocolassoc, device = "pdf", width = 7, height = 5, units = "in", dpi = 300, path = mypath)

**## Figure D - confidence intervals**

**## no systematic error**

## To find the confidence interval width around a global (combined) mean from several (k) samples of the same size with equal variance (s2). Mean values do not change the CI\_width. ## With equal variances the ratios of CI\_widths with two k values is not dependent on s.

## parameters for all (including plus systematic errors):

conf\_level <- 0.95

alpha <- 1 - conf\_level

k <- c(1, 2, 3, 4) ## number of groups

n <- 100 ## number in each group; larger n decreases differences between k2,k3,k4 (slightly !)

s <- 10 ## this is arbitrary ! changes CI\_width but doesn't change ratios of CI\_widths ## all groups have this s - this can have different values and will still give the same graph !

M <- 10 ## typical group mean - this can have different values and will still give the same graph ! used where systematic errors are added

SE <- s / sqrt(k \* n)

df <- k \* (n - 1) ## for t distribution

dist <- "normal" ## "normal" or "t" or "log\_normal"

myquantiles <- function (dist, df = NULL, alpha = 0.05) {

if (dist == "normal") {

quantiles <- qnorm(1 - alpha/2)

}

if (dist == "t") {

quantiles <- qt(1 - alpha/2, df)

}

if (dist == "log\_normal") {

quantiles <- qlnorm(1 - alpha/2)

}

return(quantiles)

}

mycrit <- function (dist, df = NULL, alpha = 0.05) {

if (dist == "normal") {

crit <- qnorm(1 - alpha/2)

}

if (dist == "t") {

crit <- qt(1 - alpha/2, df)

}

if (dist == "log\_normal") {

crit <- qlnorm(1 - alpha/2)

}

return(crit)

}

vals <- myquantiles(dist = dist, df = df, alpha = 0.05) ## df not used if normal

vals

CI\_width\_nosys\_nonAdj <- 2 \* vals \* SE

## 32.00848 25.85172 22.26758

CI\_width\_nosys <- 100 \* (CI\_width\_nosys\_nonAdj / CI\_width\_nosys\_nonAdj[[1]])

CI\_width\_nosys

## CI\_width\_nosys[[1]] / CI\_width\_nosys[[2]]

## CI\_width\_nosys[[2]] / CI\_width\_nosys[[3]]

## (CI\_width\_nosys[[1]] / CI\_width\_nosys[[2]]) - (CI\_width\_nosys[[2]] / CI\_width\_nosys[[3]])

**## Plus Uniform systematic error with range +-% of means (Uniform ±10%** and **t-distribution ±20%** give identical scaled patterns) When systematic error **scales with 1/k​** (as is realistic in many averaging scenarios), the CI narrows with more groups - **The scaled CI width curve will be identical from k >= 2**, regardless of whether the underlying distribution is uniform, normal, or log-normal.

**## Uniform distribution:** for ±20% of M:

sys\_var <- (0.20 \* M)^2 / 3 ## The variance of a continuous uniform distribution on [a,b] is known to be ((b−a)^2)/12 [Casella & Berger, 2002]. In the case of a symmetric distribution centered at zero over [−r,r], this simplifies to (r^2)/3.

sys\_var

var\_sys\_global <- sys\_var / k ## it is the 1/k here which gives the curve

var\_sys\_global

var\_sys\_global[1] <- 0 ## assumes syst. error cannot be detected for k = 1

var\_sampling <- s^2 / (k \* n)

var\_sampling

# Calculate SE including systematic error

SE\_total <- sqrt(var\_sampling + var\_sys\_global)

SE\_total

df\_total <- k \* (n - 1)

vals <- myquantiles(dist = dist, df = df\_total, alpha = 0.05) ## df not used if normal

vals

CI\_width\_1\_nonAdj <- 2 \* vals \* SE\_total

CI\_width\_1\_nonAdj

## Adjust to CI\_width\_1[[1]] as 100

CI\_width\_1 <- 100 \* CI\_width\_1\_nonAdj / CI\_width\_1\_nonAdj[[1]]

CI\_width\_1 ## with uniform distribution systematic error

# Ratios

CI\_width\_1[1] / CI\_width\_1[2]

CI\_width\_1[2] / CI\_width\_1[3]

**## Plus t-distribution systematic error scaled to % of mean**

df\_sys <- 10

systematic\_scale <- 0.15 ## % of group mean

## variance of systematic error (scaled to ±% of mean)

sigma\_sys <- M \* systematic\_scale

## Var(t\_df) = df / (df - 2), for df > 2 ## known variance for t distribution: Johnson et al. 1995.

var\_sys\_per\_group <- sigma\_sys^2 \* (df\_sys / (df\_sys - 2))

## Total variance contribution from systematic error across k groups

var\_sys\_global <- var\_sys\_per\_group / k

var\_sys\_global

var\_sys\_global[1] <- 0 ## assumes syst. error cannot be detected for k = 1

var\_sampling <- s^2 / (k \* n)

var\_sampling

SE\_total <- sqrt(var\_sampling + var\_sys\_global)

SE\_total

df <- k \* (n - 1)

crit <- mycrit(dist, df = df)

crit

CI\_width\_2\_nonAdj <- 2 \* crit \* SE\_total

CI\_width\_2\_nonAdj

## Adjust to CI\_width\_2\_nonAdj[[1]] as 100:

CI\_width\_2 <- 100 \* CI\_width\_2\_nonAdj / CI\_width\_2\_nonAdj[[1]]

CI\_width\_2 ## with t distribution systematic error

CI\_width\_2[1] / CI\_width\_2[2]

CI\_width\_2[2] / CI\_width\_2[3]

CI\_width\_1[1] / CI\_width\_1[2]

CI\_width\_1[2] / CI\_width\_1[3]

**## Plus normal distribution systematic error scaled to % of mean**

systematic\_scale <- 0.15

## Variance of systematic error (scaled to ±% of mean)

sigma\_sys <- M \* systematic\_scale

var\_sys\_per\_group <- sigma\_sys^2

# Total variance contribution from systematic error across k groups

var\_sys\_global <- var\_sys\_per\_group / k

var\_sys\_global

var\_sys\_global[1] <- 0 ## assumes syst. error cannot be detected for k = 1

var\_sampling <- s^2 / (k \* n)

var\_sampling

SE\_total <- sqrt(var\_sampling + var\_sys\_global)

SE\_total

df\_total <- k \* (n - 1)

crit <- mycrit(dist, df = df\_total)

crit

CI\_width\_3\_nonAdj <- 2 \* crit \* SE\_total

CI\_width\_3\_nonAdj

## Adjust to CI\_width\_3\_nonAdj[[1]] as 100:

CI\_width\_3 <- 100 \* CI\_width\_3\_nonAdj / CI\_width\_3\_nonAdj[[1]]

CI\_width\_3 ## with normal distribution systematic error

## For text - using only nosys values relative to k = 2 NOT k = 1 !!

nosysRelk2 <- 100 \* CI\_width\_nosys\_nonAdj / CI\_width\_nosys\_nonAdj[[2]] ## [2] !

nosysRelk2

diff1\_nosys <- nosysRelk2[1] - nosysRelk2[2]

diff2\_nosys <- nosysRelk2[2] - nosysRelk2[3]

diff3\_nosys <- nosysRelk2[3] - nosysRelk2[4]

diffs\_nosys <- c(diff1\_nosys, diff2\_nosys, diff3\_nosys)

diffs\_nosys

## 42.29623 18.51736 10.98871

## for comparison - not put onto graph:

uniRelk2 <- 100 \* CI\_width\_1\_nonAdj / CI\_width\_1\_nonAdj[[2]] ## [2] !

uniRelk2

diff1\_uni <- uniRelk2[1] - uniRelk2[2]

diff2\_uni <- uniRelk2[2] - uniRelk2[3]

diff3\_uni <- uniRelk2[3] - uniRelk2[4]

diffs\_uni <- c(diff1\_uni, diff2\_uni, diff3\_uni)

diffs\_uni

## -3.060688 18.517364 10.988709

ttRelk2 <- 100 \* CI\_width\_2\_nonAdj / CI\_width\_2\_nonAdj[[2]] ## [2] !

ttRelk2

diff1\_tt <- ttRelk2[1] - ttRelk2[2]

diff2\_tt <- ttRelk2[2] - ttRelk2[3]

diff3\_tt <- ttRelk2[3] - ttRelk2[4]

diffs\_tt <- c(diff1\_tt, diff2\_tt, diff3\_tt)

diffs\_tt

## -27.12334 18.51736 10.98871

normRelk2 <- 100 \* CI\_width\_3\_nonAdj / CI\_width\_3\_nonAdj[[2]] ## [2] !

normRelk2

diff1\_norm <- normRelk2[1] - normRelk2[2]

diff2\_norm <- normRelk2[2] - normRelk2[3]

diff3\_norm <- normRelk2[3] - normRelk2[4]

diffs\_norm <- c(diff1\_norm, diff2\_norm, diff3\_norm)

diffs\_norm

## -21.06825 18.51736 10.98871

percinc1 <- paste0(round(diff1\_nosys, 1), "%")

percinc1

percinc2 <- paste0(round(diff2\_nosys, 1), "%")

percinc2

percinc3 <- paste0(round(diff3\_nosys, 1), "%")

percinc3

CI\_width\_1 <- c(CI\_width\_1) ## with uniform distribution systematic error

CI\_width\_2 <- c(CI\_width\_2) ## with t distribution systematic error

CI\_width\_3 <- c(CI\_width\_3) ## with normal distribution systematic error

## TEMP !!

CI\_width\_1 <- c(CI\_width\_1)

CI\_width\_2 <- c(CI\_width\_2)

CI\_width\_3 <- c(CI\_width\_3)

xxx <- c("NoR", "D", "T", "T4")

xxx <- factor(xxx, levels = c("NoR", "D", "T", "T4"))

dfCI <- data.frame(xxx, CI\_width\_nosys, CI\_width\_1, CI\_width\_2, CI\_width\_3)

colnames(dfCI) <- c("Protocol", "CI\_width\_nosys", "CI\_width\_1", "CI\_width\_2", "CI\_width\_3")

cbp2 <- c("#D55E00", "#56B4E9", "#009E73", "#E69F00", "#F0E442", "#0072B2", "#CC79A7", "#000000")

mylabels <- c("No SysErr", "Uniform", "Normal", "t")

mylabels <- factor(mylabels, levels = c("No SysErr", "Uniform", "Normal", "t"))

ybreaks <- c(0, 50, 100, 140)

ylabels <- paste0(ybreaks)

ylabels

annotation1 <- data.frame(x = c(1.7, 2.4, 3.4), y = c(109, 102, 85), label = c("a", percinc2, percinc3))

annotation2 <- data.frame(x = c(1.3, 2.4, 3.4), y = c(81, 60, 49), label = c(percinc1, percinc2, percinc3))

annotation3 <- data.frame(x = c(1.39), y = c(121), label = c("b"))

dev.new()

plotCI <- ggplot(data = dfCI) +

geom\_point(aes(x = Protocol, y = CI\_width\_1, color = "Uniform"), size = 3) +

geom\_point(aes(x = Protocol, y = CI\_width\_2, color = "t"), size = 4, shape = 3) +

geom\_point(aes(x = Protocol, y = CI\_width\_3, color = "Normal"), size = 5, shape = 5) +

geom\_point(aes(x = Protocol, y = CI\_width\_nosys, color = "No SysErr"), size = 4, shape = 7) + scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 142)) + ylab("Relative C.I. width (%)") + scale\_x\_discrete(labels=c("D"="2", "T"="3", "T4"="4")) + xlab("Number of replicates (k)") + scale\_colour\_manual(name="Distribution", values = c(cbp2[8], cbp2[8], cbp2[1], cbp2[6]), breaks = mylabels, labels = mylabels) + ## breaks give correct label order

theme(legend.title = element\_text(family = "serif"), legend.position = "inside", legend.position.inside = c(0.2, 0.20), legend.box.background = element\_rect(colour = "black")) +

geom\_arrow\_segment(x = c(1, 2, 3, 10), y = c(CI\_width\_3[[1]], CI\_width\_3[[2]], CI\_width\_3[[3]], CI\_width\_3[[4]]), xend = c(2, 3, 4, 10), yend = c(CI\_width\_3[[2]], CI\_width\_3[[3]], CI\_width\_3[[4]], CI\_width\_3[[1]]), resect\_head = 2, resect\_fins = 3.5) +

geom\_arrow\_segment(x = c(1), y = 100, xend = c(1), yend = c(130), resect\_head = 5, resect\_fins = 3.5, linetype = "dashed", linewidth = 0.7) +

geom\_arrow\_segment(x = c(1, 2, 3, 10), y = c(CI\_width\_2[[1]], CI\_width\_2[[2]], CI\_width\_2[[3]], CI\_width\_2[[4]]), xend = c(2, 3, 4, 10), yend = c(CI\_width\_2[[2]], CI\_width\_2[[3]], CI\_width\_2[[4]], CI\_width\_2[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "dashed", linewidth = 0.7) +

geom\_arrow\_segment(x = c(1, 2, 3, 10), y = c(CI\_width\_nosys[[1]], CI\_width\_nosys[[2]], CI\_width\_nosys[[3]], CI\_width\_nosys[[4]]), xend = c(2, 3, 4, 10), yend = c(CI\_width\_nosys[[2]], CI\_width\_nosys[[3]], CI\_width\_nosys[[4]], CI\_width\_nosys[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "dashed", linewidth = 0.7) +

geom\_arrow\_segment(x = c(1, 2, 3, 10), y = c(CI\_width\_1[[1]], CI\_width\_1[[2]], CI\_width\_1[[3]], CI\_width\_1[[4]]), xend = c(2, 3, 4, 10), yend = c(CI\_width\_1[[2]], CI\_width\_1[[3]], CI\_width\_1[[4]], CI\_width\_1[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "dashed", linewidth = 0.7) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation2, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation3, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold")

one\_slice <- data.frame(start = 0.1, end = 0.77\*pi, r0 = 0, r = 10)

p\_circle <- ggplot(one\_slice) +

ggforce::geom\_arc\_bar(aes(x0 = 0, y0 = 100, r0 = 0, r = 10, start = start, end = end),

fill = cbp2[2], alpha = 0.5, colour = cbp2[2], linewidth = 1, n = 180) +

coord\_fixed(xlim = c(0, 4), ylim = c(0, 140), clip = "off") +

theme\_void()

library(grid)

plotCIplus <- cowplot::ggdraw() +

cowplot::draw\_plot(plotCI) +

draw\_plot(p\_circle, x = -0.187, y = 0.015, width = 1, height = 1)

dev.new(width = 6, height = 7)

print(plotCIplus)

ggsave("FigureD\_CI.pdf", plot = plotCIplus, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

## ## for main parameter t distribution, n = 100:

## > diff1

## [1] 18.51736

## > diff2

## [1] 10.98871

## for main parameter t distribution, n = 1000000:

## > diff1

## [1] 18.35036

## > diff2

## [1] 10.93898

## ## for main parameter normal or log\_normal distribution:

## > diff1

## [1] 18.35034

## > diff2

## [1] 10.93898

##

**## Figure 5B - confidence intervals with true values at k=1:**

mylabels <- c("t", "Normal", "Uniform", "No SysErr")

mylabels <- factor(mylabels, levels = c("t", "Normal", "Uniform", "No SysErr"))

trueProInc1 <- 1 + diff1\_nosys / 100

trueProInc1

trueCI <- trueProInc1\* c(CI\_width\_nosys[[2]], CI\_width\_1[[2]], CI\_width\_3[[2]], CI\_width\_2[[2]])

names(trueCI) <- c("No SysErr", "Uniform", "Normal", "t")

trueCI

CI\_width\_nosystrue <- CI\_width\_nosys ## no SysErr

CI\_width\_nosystrue[[1]] <- trueCI[["No SysErr"]]

CI\_width\_nosystrue

CI\_width\_1true <- CI\_width\_1 ## with uniform distribution systematic error

CI\_width\_1true[[1]] <- trueCI[["Uniform"]]

CI\_width\_1true

CI\_width\_2true <- CI\_width\_2 ## with t distribution systematic error

CI\_width\_2true[[1]] <- trueCI[["t"]]

CI\_width\_2true

CI\_width\_3true <- CI\_width\_3 ## with normal distribution systematic error

CI\_width\_3true[[1]] <- trueCI[["Normal"]]

CI\_width\_3true

Protocol <- dfCI[ , "Protocol"]

Protocol

dfCItrue <- data.frame(Protocol, CI\_width\_nosystrue, CI\_width\_1true, CI\_width\_2true, CI\_width\_3true)

dfCItrue

ybreaks <- c(0, 50, 100, 150, 200)

ylabels <- paste0(ybreaks)

ylabels

annotation1 <- data.frame(x = c(1.5, 2.4, 3.4), y = c(109, 94, 78), label = c("a", percinc2, percinc3))

annotation2 <- data.frame(x = c(1.3, 1.3, 2.4, 3.4), y = c(81, 140, 60, 49), label = c(percinc1, percinc1, percinc2, percinc3))

annotation3 <- data.frame(x = c(1.39), y = c(121), label = c("b"))

plotCItrue <- ggplot(data = dfCI) +

geom\_point(aes(x = Protocol, y = CI\_width\_nosys, color = "No SysErr"), size = 4, shape = 7) +

geom\_point(aes(x = Protocol, y = CI\_width\_1, color = "Uniform"), size = 3) +

geom\_point(aes(x = Protocol, y = CI\_width\_2, color = "t"), size = 4, shape = 3) +

geom\_point(aes(x = Protocol, y = CI\_width\_3, color = "Normal"), size = 5, shape = 5) +

geom\_point(data = dfCItrue[1, ], aes(x = Protocol, y = CI\_width\_nosystrue, color = "No SysErr"), size = 4, shape = 7) +

geom\_point(data = dfCItrue[1, ], aes(x = Protocol, y = CI\_width\_1true, color = "Uniform"), size = 3) +

geom\_point(data = dfCItrue[1, ], aes(x = Protocol, y = CI\_width\_2true, color = "t"), size = 4, shape = 3) +

geom\_point(data = dfCItrue[1, ], aes(x = Protocol, y = CI\_width\_3true, color = "Normal"), size = 5, shape = 5) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 200)) + ylab("Relative C.I. width (%)") + scale\_x\_discrete(labels=c("D"="2", "T"="3", "T4"="4")) + xlab("Number of replicates (k)") + scale\_colour\_manual(name="SysErr distribution", values = c(cbp2[8], cbp2[8], cbp2[1], cbp2[6]), breaks = mylabels, labels = mylabels) + ## breaks give correct label order

theme(legend.title = element\_text(family = "serif"), legend.position = "inside", legend.position.inside = c(0.8, 0.8), legend.box.background = element\_rect(colour = "black")) + discrete\_scale("linetype", palette = linetypes) +

geom\_segment(x = c(1, 2, 3, 10), y = c(CI\_width\_2[[1]], CI\_width\_2[[2]], CI\_width\_2[[3]], CI\_width\_2[[4]]), xend = c(2, 3, 4, 10), yend = c(CI\_width\_2[[2]], CI\_width\_2[[3]], CI\_width\_2[[4]], CI\_width\_2[[1]]), linetype = "twodash", linewidth = 0.7) + **## t lines**

geom\_arrow\_segment(x = c(2, 3, 10, 20), y = c(CI\_width\_3[[2]], CI\_width\_3[[3]], CI\_width\_3[[4]], CI\_width\_3[[1]]), xend = c(3, 4, 10, 20), yend = c(CI\_width\_3[[3]], CI\_width\_3[[4]], CI\_width\_3[[1]], CI\_width\_3[[2]]), resect\_head = 2, resect\_fins = 3.5, linetype = "solid") + **## Normal arrows**

geom\_segment(x = c(1), y = c(CI\_width\_3[[1]]), xend = c(2), yend = c(CI\_width\_3[[2]]), linetype = "solid", linewidth = 1) + **## Normal line**

geom\_segment(x = c(1), y = 100, xend = c(1), yend = c(130), linetype = "dashed", linewidth = 0.7) + **## vertical arrow**

geom\_arrow\_segment(x = c(2, 3, 10, 20), y = c(CI\_width\_nosys[[2]], CI\_width\_nosys[[3]], CI\_width\_nosys[[4]], CI\_width\_nosys[[4]]), xend = c(3, 4, 10, 20), yend = c(CI\_width\_nosys[[3]], CI\_width\_nosys[[4]], CI\_width\_nosys[[1]], CI\_width\_nosys[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "solid", linewidth = 0.7) + **## No SysErr arrows**

geom\_arrow\_segment(x = c(2), y = c(CI\_width\_nosys[[2]]), xend = c(1), yend = c(CI\_width\_nosys[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "solid", linewidth = 0.7) + **## No SysErr arrows**

geom\_arrow\_segment(x = c(2, 3, 10, 20), y = c(CI\_width\_1[[2]], CI\_width\_1[[3]], CI\_width\_1[[4]], CI\_width\_1[[4]]), xend = c(3, 4, 10, 20), yend = c(CI\_width\_1[[3]], CI\_width\_1[[4]], CI\_width\_1[[1]], CI\_width\_1[[1]]), resect\_head = 2, resect\_fins = 3.5, linetype = "solid", linewidth = 0.7) + **## Uniform arrows**

geom\_segment(x = c(1), y = c(CI\_width\_1[[1]]), xend = c(2), yend = c(CI\_width\_1[[2]]), linetype = "solid", linewidth = 0.7) + **## Uniform line**

geom\_segment(data = dfCItrue, x = c(1), y = c(dfCItrue[1, "CI\_width\_2true"]), xend = c(2), yend = c(dfCItrue[2, "CI\_width\_2true"]), linetype = "twodash", linewidth = 0.7) + **## twodash line t**

geom\_arrow\_segment(data = dfCItrue, x = c(2), y = c(dfCItrue[2, "CI\_width\_1true"]), xend = c(1), yend = c(dfCItrue[1, "CI\_width\_1true"]), resect\_head = 2, resect\_fins = 3.5, linetype = "19", linewidth = 0.7) + **## dotted arrow Uniform**

geom\_arrow\_segment(data = dfCItrue, x = c(2), y = dfCItrue[2, "CI\_width\_3true"], xend = c(1), yend = c(dfCItrue[1, "CI\_width\_3true"]), resect\_head = 2, resect\_fins = 3.5, linetype = "19", linewidth = 0.7) + **## dotted arrow Normal**

geom\_segment(data = dfCItrue, x = c(2), y = 0, xend = c(2), yend = dfCItrue[2, "CI\_width\_nosystrue"], linetype = "19", linewidth = 0.7, color = cbp2[1]) + **## vertical line k=2**

geom\_segment(data = dfCItrue, x = c(3), y = 0, xend = c(3), yend = dfCItrue[3, "CI\_width\_nosystrue"], linetype = "19", linewidth = 0.7, color = cbp2[1]) + **## vertical line k=3**

geom\_segment(data = dfCItrue, x = c(4), y = 0, xend = c(4), yend = dfCItrue[4, "CI\_width\_nosystrue"], linetype = "19", linewidth = 0.7, color = cbp2[1]) + **## vertical line k=4**

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation2, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

annotate("text", x = 1.8, y = 25, label = "sqrt(1/2)", parse = TRUE, color = cbp2[1],

fontface = "bold") +

annotate("text", x = 2.8, y = 25, label = "sqrt(2/3)", parse = TRUE, color = cbp2[1],

fontface = "bold") +

annotate("text", x = 3.8, y = 25, label = "sqrt(3/4)", parse = TRUE, color = cbp2[1],

fontface = "bold")

one\_slice <- data.frame(start = 0.05, end = 0.75\*pi, r0 = 0, r = 10) ## edge angles of arc

p\_circle <- ggplot(one\_slice) +

ggforce::geom\_arc\_bar(aes(x0 = 0, y0 = 100, r0 = 0, r = 10, start = start, end = end),

fill = cbp2[2], alpha = 0.5, colour = cbp2[2], linewidth = 1, n = 180) +

coord\_fixed(xlim = c(0, 4), ylim = c(0, 200), clip = "off") +

theme\_void()

library(grid)

plotCIplus <- cowplot::ggdraw() +

cowplot::draw\_plot(plotCItrue) +

draw\_plot(p\_circle, x = -0.21, y = 0.037, width = 1, height = 1) ## point of arc adjust

plotwidth <- 7

plotheight <- 9

dev.new(width = plotwidth, height = plotheight)

print(plotCIplus)

ggsave("FigureDB\_CI.pdf", plot = plotCIplus, device = "pdf", width = plotwidth, height = plotheight, units = "in", dpi = 300, path = mypath)

**## Plus t-distribution systematic error - WITH VARYING N:**

set.seed(123)

conf\_level <- 0.95

alpha <- 1 - conf\_level

k <- c(2, 3, 4)

n <- c(2, 10, 33, 100, 333, 10000)

kdf <- data.frame(matrix(k, ncol = length(k), nrow = length(n), byrow = TRUE))

head(kdf)

ndf <- kdf

for (i in 1:length(k)) {

ndf[ , i] <- n

}

colnames(ndf) <- k

colnames(kdf) <- k

head(ndf)

head(kdf)

s <- 50 ## all groups have this s - this can have different values and will still give the same graph !

M <- 10 ## typical group mean - this can have different values and will still give the same graph !

df\_sys <- 30 ## degrees of freedom for t-distribution

systematic\_scale <- 0.10 ## 10% of group mean

## Variance of systematic error (scaled to ±10% of mean)

sigma\_sys <- M \* systematic\_scale

## Var(t\_df) = df / (df - 2), for df > 2

var\_sys\_per\_group <- sigma\_sys^2 \* (df\_sys / (df\_sys - 2))

var\_sys\_global <- var\_sys\_per\_group / k

var\_sys\_global

var\_sampling <- s^2 / (kdf \* ndf)

head(var\_sampling, 100)

SE\_total <- var\_sampling

for (i in 1:nrow(SE\_total)) {

SE\_total[i, ] <- sqrt(var\_sampling[i, ] + var\_sys\_global)

}

head(SE\_total)

df\_total <- kdf \* (ndf - 1)

head(df\_total)

crit <- df\_total; ## temp for structure

for (i in 1:ncol(df\_total)) {

for (j in 1:nrow(df\_total)) {

crit[j, i] <- qt(1 - alpha/2, df\_total[j, i])

}  
}  
head(crit)

CI\_width\_n\_nonAdj <- 2 \* crit \* SE\_total

head(CI\_width\_n\_nonAdj)

## Adjust to CI\_width\_n\_nonAdj[ , 1] as 100:

CI\_width\_n <- CI\_width\_n\_nonAdj

for (i in 1:ncol(CI\_width\_n)) {

CI\_width\_n[ , i] <- CI\_width\_n\_nonAdj[ , i] / CI\_width\_n\_nonAdj[ , 1]

}

head(CI\_width\_n)

CI\_width\_n <- CI\_width\_n \* 100

head(CI\_width\_n)

CI\_width\_n <- as.data.frame(t(CI\_width\_n))

colnames(CI\_width\_n) <- n

CI\_width\_n <- cbind(k, CI\_width\_n)

head(CI\_width\_n)

n\_char <- as.character(n)

n\_char <- factor(n\_char, levels = unique(n\_char))

mylabels <- paste0(n\_char)

mylabels[6] <- "10000 or normal"

dev.new()

plotCInA <- ggplot(data = CI\_width\_n) +

geom\_point(aes(x = k, CI\_width\_n[ , 2], color = n\_char[[1]]), size = 3, shape = 1) +

geom\_point(aes(x = k, y = CI\_width\_n[ , 3], color = n\_char[[2]]), size = 6, shape = 3) +

geom\_point(aes(x = k, y = CI\_width\_n[ , 4], color = n\_char[[3]]), size = 4, shape = 17) +

geom\_point(aes(x = k, y = CI\_width\_n[ , 5], color = n\_char[[4]]), size = 4, shape = 4) +

geom\_point(aes(x = k, y = CI\_width\_n[ , 6], color = n\_char[[5]]), size = 4, shape = 18) +

geom\_point(aes(x = k, y = CI\_width\_n[ , 7], color = n\_char[[6]]), size = 4, shape = 6) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 2], color = n\_char[[1]])) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 3], color = n\_char[[2]])) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 4], color = n\_char[[3]])) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 5], color = n\_char[[4]])) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 6], color = n\_char[[5]])) +

geom\_line(aes(x = k, y = CI\_width\_n[ , 7], color = n\_char[[6]])) + scale\_x\_continuous(breaks = c(2, 3, 4), labels = c("2", "3", "4"), expand = c(0, 0), limits = c(1.8, 4.2)) + scale\_y\_continuous(breaks = c(0, 20, 40, 60, 80, 100), labels = c("0", "20", "40", "60", "80", "100"), expand = c(0, 0), limits = c(0, 105)) + ylab("Relative C.I. width (%)") + xlab("Number of replicates") +

scale\_colour\_manual(name = "Sample size, one rep", values = c(cbp2[1], cbp2[8], cbp2[7], cbp2[6], cbp2[5], cbp2[4]), limits = c(n\_char[[1]], n\_char[[2]], n\_char[[3]], n\_char[[4]], n\_char[[5]], n\_char[[6]]), labels = mylabels) + theme(legend.title = element\_text(family = "serif"), legend.position = "inside", legend.position.inside = c(0.29, 0.27), legend.box.background = element\_rect(colour = "black"))

## Add label to plot:

plotCIn <- cowplot::ggdraw(plotCInA) + draw\_figure\_label(label = "a.", position = c("top.left"), size = 25, fontface = "bold")

plot(plotCIn)

ggsave("FigureE\_CIn.pdf", plot = plotCIn, device = "pdf", width = 6, height = 6, units = "in", dpi = 300, path = mypath)

**## Plus t-distribution systematic error - Gain versus N:**

conf\_level <- 0.95

alpha <- 1 - conf\_level

k <- c(2, 3, 4)

n <- seq(from = 5, to = 335, by = 5)

kdf <- data.frame(matrix(k, ncol = length(k), nrow = length(n), byrow = TRUE))

head(kdf)

ndf <- kdf

for (i in 1:length(k)) {

ndf[ , i] <- n

}

colnames(ndf) <- k

colnames(kdf) <- k

head(ndf)

head(kdf)

s <- 50 ## all groups have this s - this can have different values and will still give the same graph !

M <- 10 ## typical group mean - this can have different values and will still give the same graph !

df\_sys <- 30 # degrees of freedom for t-distribution

systematic\_scale <- 0.10 # 10% of group mean

## Variance of systematic error (scaled to ±10% of mean)

sigma\_sys <- M \* systematic\_scale

## Var(t\_df) = df / (df - 2), for df > 2

var\_sys\_per\_group <- sigma\_sys^2 \* (df\_sys / (df\_sys - 2))

var\_sys\_global <- var\_sys\_per\_group / k

var\_sys\_global

var\_sampling <- s^2 / (kdf \* ndf)

head(var\_sampling, 100)

SE\_total <- var\_sampling

for (i in 1:nrow(SE\_total)) {

SE\_total[i, ] <- sqrt(var\_sampling[i, ] + var\_sys\_global)

}

head(SE\_total)

df\_total <- kdf \* (ndf - 1)

head(df\_total)

crit <- df\_total;

for (i in 1:ncol(df\_total)) {

for (j in 1:nrow(df\_total)) {

crit[j, i] <- qt(1 - alpha/2, df\_total[j, i])

}  
}  
head(crit)

CI\_width\_ng\_nonAdj <- 2 \* crit \* SE\_total

head(CI\_width\_ng\_nonAdj)

## Adjust to CI\_width\_ng\_nonAdj[ , 1] as 100:

CI\_width\_ng <- CI\_width\_ng\_nonAdj

for (i in 1:ncol(CI\_width\_ng)) {

CI\_width\_ng[ , i] <- CI\_width\_ng\_nonAdj[ , i] / CI\_width\_ng\_nonAdj[ , 1]

}

head(CI\_width\_ng)

CI\_width\_ng$diff1 <- 100 \* (CI\_width\_ng[ , 1] - CI\_width\_ng[ , 2])

CI\_width\_ng$diff2 <- 100 \* (CI\_width\_ng[ , 2] - CI\_width\_ng[ , 3])

CI\_width\_ng$Gain <- 100 \* (CI\_width\_ng$diff1 - CI\_width\_ng$diff2) / CI\_width\_ng$diff2

CI\_width\_ng$n <- n

head(CI\_width\_ng)

class(CI\_width\_ng)

nrow(CI\_width\_ng)

ncol(CI\_width\_ng)

dev.new()

plotGainA <- ggplot(data = CI\_width\_ng) +

geom\_line(aes(x = n, y = Gain)) + scale\_y\_continuous(breaks = c(0, 10, 30, 50, 70, 90, 100), labels = c("0", "10", "30", "50", "70", "90", "100"), expand = c(0, 0), limits = c(0, 100)) + ylab("Gain (%)") + xlab("Sample size (n) per replicate") + coord\_cartesian(xlim = c(0, 300), clip = 'off')

plotGainA

plotGain <- cowplot::ggdraw(plotGainA) + draw\_figure\_label(label = "b.", position = c("top.left"), size = 25, fontface = "bold")

plotGain

ggsave("FigureF\_Gain.pdf", plot = plotGain, device = "pdf", width = 6, height = 6, units = "in", dpi = 300, path = mypath)

mymin <- CI\_width\_ng[CI\_width\_ng$Gain == min(CI\_width\_ng$Gain), ]

mymin

## 2 3 4 diff1 diff2 Gain n

## 67 1 0.8160029 0.7064656 18.39971 10.95372 67.97677 335

**## SYSTEMATIC ERRORS FROM DATA:**

**## REAL WORLD DATA**

## 17 Sotelo Montes Table 2 Region 1 Sp\_MOE-Tan

## Statistically significant differences between means shown - but variances not given.

## 28 Flores\_Fernandez Table 2 Undisturbed control plots - s.d. not given

## 40 Koehler emergence rates Table 4

## Statistically significant differences between means shown - but variances not given

## Brzostowski et al. 2017 Table 1 Seed yield

## Statistically significant differences between means shown - but variances not given

## Wang et al. 2017 Fig 8 D

## Results were repeated three times - but representative results shown

## Ruxton 2006

## SE is sp / sqrt(n \* k) ## SE is NOT sp / sqrt(n/k) = sp \* sqrt(k/n) which would be for analyzing differences

## The degrees of freedom for unequal variances were estimated using the Welch–Satterthwaite approximation (as noted by the International Organization for Standardization: ISO Guide to the Expression of Uncertainty in Measurement):

## Ballico 2000

## Welch 1947

**## First part assumes NO SYSTEMATIC ERRORS FROM DATA:**

global\_mean\_CI\_nosys <- function(means, ss, s\_method = c("sd", "SE"), ns, alpha = 0.05) {

s\_method <- match.arg(s\_method)

k <- length(means)

total\_n <- sum(ns)

weights <- ns / total\_n

global\_mean <- sum(weights \* means)

if (s\_method == "sd") {

sds <- ss

var\_total <- sum((sds^2 / ns) \* (weights^2))

var\_i <- (weights^2 \* sds^2) / ns

} else if (s\_method == "SE") {

SE <- ss

var\_total <- sum((weights^2) \* (SE^2))

var\_i <- (weights^2 \* SE^2)

}

SE\_total <- sqrt(var\_total)

df\_num <- var\_total^2

df\_den <- sum(var\_i^2 / (ns - 1)) ## Welch–Satterthwaite df correction - differing variances

df <- df\_num / df\_den

t\_crit <- qt(p = (1 - alpha/2), df)

CI\_lower <- global\_mean - t\_crit \* SE\_total

CI\_upper <- global\_mean + t\_crit \* SE\_total

CI\_width <- 2\*t\_crit \* SE\_total

CI\_expected <- qt((1-alpha/2), df = sum(ns-1)) / sqrt(k)

return(c(CI\_width = CI\_width, CI\_expected = CI\_expected))

}

## C.I. width ratios relative to k = 2

ybreaks <- c(0, 100, 200)

ylabels <- paste0(ybreaks)

ylabels

**## A. Tonk et al. 2017 Fig 1. (a) top panel Landraces TR1 to TR7**

Tonk\_label <- c("TR1", "TR2", "TR3", "TR4", "TR5", "TR6", "TR7")

Tonk\_mean <- c(34, 52, 46, 56, 39, 49, 23.5)

Tonk\_sd <- c(8.75, 15.25, 12.5, 25, 10.75, 15.25, 4.5)

Tonk\_n <- c(100, 100, 100, 100, 100, 100, 100)

Tonk\_raw\_df <- data.frame(Tonk\_label, Tonk\_mean, Tonk\_sd, Tonk\_n)

Tonk\_raw\_df

colnames(Tonk\_raw\_df) <- c("label", "mean", "sd", "n")

Tonk\_raw\_df

k <- 1:7

n <- 100

Tonk\_CIdata <- list();

for (i in 1:nrow(Tonk\_raw\_df)) {

Tonk\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Tonk\_mean[1:i], ss = Tonk\_sd[1:i], s\_method = "sd", ns = Tonk\_n[1:i])

}

Tonk\_CIdf <- as.data.frame(list.rbind(Tonk\_CIdata))

Tonk\_CIdf

Tonk\_actual\_ratio <- 100 \* signif(Tonk\_CIdf$CI\_width / Tonk\_CIdf[2, "CI\_width"], 4)

Tonk\_actual\_ratio

Tonk\_expected\_ratio <- 100 \* signif(Tonk\_CIdf$CI\_expected / Tonk\_CIdf[2, "CI\_expected"], 4)

Tonk\_expected\_ratio

Tonkdf <- data.frame(k, Tonk\_actual\_ratio, Tonk\_expected\_ratio)

colnames(Tonkdf) <- c("k", "Tonk\_Act", "Tonk\_Exp")

Tonkdf

plotRealTonk <- ggplot(data = Tonkdf) +

geom\_point(aes(x = k, y = Tonk\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Tonk\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = c(1, 3, 5, 7), expand = c(0, 0), limits = c(1, max(k) + 0.5)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Tonk\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Tonk\_Exp, color = "Expected"))

dev.new()

plotRealTonk

ggsave("FigureG\_Tonk\_real.pdf", plot = plotRealTonk, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## B. Kichigina et al. 2017 Fig 5(a) - Al sensitive genotypes**

Kich\_label <- c("3654", "8473", "2759", "1903")

Kich\_mean <- c(2.4, 3.9, 1.4, 7)

Kich\_SE <- c(0.35, 1.1, 0.15, 1.1)

Kich\_n <- c(5, 5, 5, 5)

Kich\_raw\_df <- data.frame(Kich\_label, Kich\_mean, Kich\_SE, Kich\_n)

Kich\_raw\_df

colnames(Kich\_raw\_df) <- c("label", "mean", "SE", "n")

Kich\_raw\_df

k <- 1:4

n <- 5

Kich\_CIdata <- list();

for (i in 1:nrow(Kich\_raw\_df)) {

Kich\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Kich\_mean[1:i], ss = Kich\_SE[1:i], s\_method = "SE", ns = Kich\_n[1:i])

}

Kich\_CIdf <- as.data.frame(list.rbind(Kich\_CIdata))

Kich\_CIdf

Kich\_actual\_ratio <- 100 \* signif(Kich\_CIdf$CI\_width / Kich\_CIdf[2, "CI\_width"], 4)

Kich\_actual\_ratio

Kich\_expected\_ratio <- 100 \* signif(Kich\_CIdf$CI\_expected / Kich\_CIdf[2, "CI\_expected"], 4)

Kich\_expected\_ratio

Kichdf <- data.frame(k, Kich\_actual\_ratio, Kich\_expected\_ratio)

colnames(Kichdf) <- c("k", "Kich\_Act", "Kich\_Exp")

Kichdf

plotRealKich <- ggplot(data = Kichdf) +

geom\_point(aes(x = k, y = Kich\_Act), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Kich\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.y = element\_blank()) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Kich\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Kich\_Exp, color = "Expected"))

dev.new()

plotRealKich

ggsave("FigureG\_Kich\_real.pdf", plot = plotRealKich, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## C. Carlucci et al. 2017 Table 5 Ilyonectria liriodendri**

Carl\_label <- c("BF144", "BF47", "BF12")

Carl\_mean <- c(8.2, 10.8, 11.8)

Carl\_sd <- c(3.1, 1.1, 1.9)

Carl\_n <- c(10, 10, 10)

Carl\_raw\_df <- data.frame(Carl\_label, Carl\_mean, Carl\_sd, Carl\_n)

Carl\_raw\_df

colnames(Carl\_raw\_df) <- c("label", "mean", "sd", "n")

Carl\_raw\_df

## N is a guess, but note the patterns of relative C.I. do not change substantially from N=2 to N=10000 in terms of Actual being above Expected for k=1 (both are shifted upwards for N=2 or downwards slightly for N=10000)

k <- 1:3

n <- 10

Carl\_CIdata <- list();

for (i in 1:nrow(Carl\_raw\_df)) {

Carl\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Carl\_mean[1:i], ss = Carl\_sd[1:i], s\_method = "sd", ns = Carl\_n[1:i])

}

Carl\_CIdf <- as.data.frame(list.rbind(Carl\_CIdata))

Carl\_CIdf

Carl\_actual\_ratio <- 100 \* signif(Carl\_CIdf$CI\_width / Carl\_CIdf[2, "CI\_width"], 4)

Carl\_actual\_ratio

Carl\_expected\_ratio <- 100 \* signif(Carl\_CIdf$CI\_expected / Carl\_CIdf[2, "CI\_expected"], 4)

Carl\_expected\_ratio

Carldf <- data.frame(k, Carl\_actual\_ratio, Carl\_expected\_ratio)

colnames(Carldf) <- c("k", "Carl\_Act", "Carl\_Exp")

Carldf

Carldf <- data.frame(k, Carl\_actual\_ratio, Carl\_expected\_ratio)

colnames(Carldf) <- c("k", "Carl\_Act", "Carl\_Exp")

Carldf

plotRealCarl <- ggplot(data = Carldf) +

geom\_point(aes(x = k, y = Carl\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Carl\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.y = element\_blank()) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Carl\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Carl\_Exp, color = "Expected"))

dev.new()

plotRealCarl

ggsave("FigureG\_Carl\_real.pdf", plot = plotRealCarl, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## D. Lawrence2017 - FIG 1 NATIVE**

Lawr\_label <- c("Gardener Marsh", "Pheasant Branch Slough", "Prairie Wolf")

Lawr\_mean <- c(6.2, 5.3, 14.2)

Lawr\_SE <- c(2.1, 3, 3.9)

Lawr\_n <- c(5, 5, 5)

Lawr\_raw\_df <- data.frame(Lawr\_label, Lawr\_mean, Lawr\_SE, Lawr\_n)

Lawr\_raw\_df

colnames(Lawr\_raw\_df) <- c("label", "mean", "SE", "n")

Lawr\_raw\_df

k <- 1:3

n <- 5

Lawr\_CIdata <- list();

for (i in 1:nrow(Lawr\_raw\_df)) {

Lawr\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Lawr\_mean[1:i], ss = Lawr\_SE[1:i], s\_method = "SE", ns = Lawr\_n[1:i])

}

Lawr\_CIdf <- as.data.frame(list.rbind(Lawr\_CIdata))

Lawr\_CIdf

Lawr\_actual\_ratio <- 100 \* signif(Lawr\_CIdf$CI\_width / Lawr\_CIdf[2, "CI\_width"], 4)

Lawr\_actual\_ratio

Lawr\_expected\_ratio <- 100 \* signif(Lawr\_CIdf$CI\_expected / Lawr\_CIdf[2, "CI\_expected"], 4)

Lawr\_expected\_ratio

Lawrdf <- data.frame(k, Lawr\_actual\_ratio, Lawr\_expected\_ratio)

colnames(Lawrdf) <- c("k", "Lawr\_Act", "Lawr\_Exp")

Lawrdf

plotRealLawr <- ggplot(data = Lawrdf) +

geom\_point(aes(x = k, y = Lawr\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Lawr\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") +

geom\_line(aes(x = k, y = Lawr\_Act, color = "Actual")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Lawr\_Exp, color = "Expected"))

dev.new()

plotRealLawr

ggsave("FigureG\_Lawr\_real.pdf", plot = plotRealLawr, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## E. Zhang et al. 2017 Fig 8 A Control**

Zhan\_label <- c("Merlot", "Syrah", "Concord")

Zhan\_mean <- c(20, 23.5, 27.2)

Zhan\_SE <- c(1, 1, 1.4)

Zhan\_n <- c(10, 10, 10)

Zhan\_raw\_df <- data.frame(Zhan\_label, Zhan\_mean, Zhan\_SE, Zhan\_n)

Zhan\_raw\_df

colnames(Zhan\_raw\_df) <- c("label", "mean", "SE", "n")

Zhan\_raw\_df

k <- 1:3

n <- 10

Zhan\_CIdata <- list();

for (i in 1:nrow(Zhan\_raw\_df)) {

Zhan\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Zhan\_mean[1:i], ss = Zhan\_SE[1:i], s\_method = "SE", ns = Zhan\_n[1:i])

}

Zhan\_CIdf <- as.data.frame(list.rbind(Zhan\_CIdata))

Zhan\_CIdf

Zhan\_actual\_ratio <- 100 \* signif(Zhan\_CIdf$CI\_width / Zhan\_CIdf[2, "CI\_width"], 4)

Zhan\_actual\_ratio

Zhan\_expected\_ratio <- 100 \* signif(Zhan\_CIdf$CI\_expected / Zhan\_CIdf[2, "CI\_expected"], 4)

Zhan\_expected\_ratio

Zhan\_actual\_ratio <- c(152.3, 100, 91.62)

Zhan\_expected\_ratio <- c(152.3, 100, 79.74)

Zhandf <- data.frame(k, Zhan\_actual\_ratio, Zhan\_expected\_ratio)

colnames(Zhandf) <- c("k", "Zhan\_Act", "Zhan\_Exp")

Zhandf

plotRealZhan <- ggplot(data = Zhandf) +

geom\_point(aes(x = k, y = Zhan\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Zhan\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.y = element\_blank()) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Zhan\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Zhan\_Exp, color = "Expected"))

dev.new()

plotRealZhan

ggsave("FigureG\_Zhan\_real.pdf", plot = plotRealZhan, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## F. Driver et al. 2017 Fig 9B, Total PA, High P**

Driv\_label <- c("GPD3-OE1", "GPD3-OE2", "GPD3-OE3")

Driv\_mean <- c(1.8, 2.6, 3)

Driv\_SE <- c(0.4, 0.6, 1.3)

Driv\_n <- c(3, 3, 3)

Driv\_raw\_df <- data.frame(Driv\_label, Driv\_mean, Driv\_SE, Driv\_n)

Driv\_raw\_df

colnames(Driv\_raw\_df) <- c("label", "mean", "SE", "n")

Driv\_raw\_df

k <- 1:3

n <- 3

Driv\_CIdata <- list();

for (i in 1:nrow(Driv\_raw\_df)) {

Driv\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Driv\_mean[1:i], ss = Driv\_SE[1:i], s\_method = "SE", ns = Driv\_n[1:i])

}

Driv\_CIdf <- as.data.frame(list.rbind(Driv\_CIdata))

Driv\_CIdf

Driv\_actual\_ratio <- 100 \* signif(Driv\_CIdf$CI\_width / Driv\_CIdf[2, "CI\_width"], 4)

Driv\_actual\_ratio

Driv\_expected\_ratio <- 100 \* signif(Driv\_CIdf$CI\_expected / Driv\_CIdf[2, "CI\_expected"], 4)

Driv\_expected\_ratio

Drivdf <- data.frame(k, Driv\_actual\_ratio, Driv\_expected\_ratio)

colnames(Drivdf) <- c("k", "Driv\_Act", "Driv\_Exp")

Drivdf

plotRealDriv <- ggplot(data = Drivdf) +

geom\_point(aes(x = k, y = Driv\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Driv\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.y = element\_blank()) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Driv\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Driv\_Exp, color = "Expected"))

dev.new()

plotRealDriv

ggsave("FigureG\_Driv\_real.pdf", plot = plotRealDriv, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## G. Zhou et al. 2017 Fig 3A Relative transcription level**

Zhou\_label <- c("RG2", "RG6", "RG14", "RG20")

Zhou\_mean <- c(0.8, 0.9, 0.8, 2.1)

Zhou\_sd <- c(0.3, 0.35, 0.5, 0.5)

Zhou\_n <- c(3, 3, 3, 3)

Zhou\_raw\_df <- data.frame(Zhou\_label, Zhou\_mean, Zhou\_sd, Zhou\_n)

Zhou\_raw\_df

colnames(Zhou\_raw\_df) <- c("label", "mean", "sd", "n")

Zhou\_raw\_df

k <- 1:4

n <- 3

Zhou\_CIdata <- list();

for (i in 1:nrow(Zhou\_raw\_df)) {

Zhou\_CIdata[[i]] <- global\_mean\_CI\_nosys(means = Zhou\_mean[1:i], ss = Zhou\_sd[1:i], s\_method = "sd", ns = Zhou\_n[1:i])

}

Zhou\_CIdf <- as.data.frame(list.rbind(Zhou\_CIdata))

Zhou\_CIdf

Zhou\_actual\_ratio <- 100 \* signif(Zhou\_CIdf$CI\_width / Zhou\_CIdf[2, "CI\_width"], 4)

Zhou\_actual\_ratio

Zhou\_expected\_ratio <- 100 \* signif(Zhou\_CIdf$CI\_expected / Zhou\_CIdf[2, "CI\_expected"], 4)

Zhou\_expected\_ratio

Zhoudf <- data.frame(k, Zhou\_actual\_ratio, Zhou\_expected\_ratio)

colnames(Zhoudf) <- c("k", "Zhou\_Act", "Zhou\_Exp")

Zhoudf

plotRealZhou <- ggplot(data = Zhoudf) +

geom\_point(aes(x = k, y = Zhou\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Zhou\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = k, expand = c(0, 0), limits = c(1, max(k) + 0.2)) + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

theme(legend.position = "none") + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Zhou\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Zhou\_Exp, color = "Expected"))

dev.new()

plotRealZhou

ggsave("FigureG\_Zhou\_real.pdf", plot = plotRealZhou, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## All Real data:**

mylist <- list(Tonkdf, Kichdf, Carldf, Lawrdf, Zhandf, Drivdf, Zhoudf)

mylist

plotlist <- list(plotRealTonk, plotRealKich, plotRealCarl, plotRealLawr, plotRealZhan, plotRealDriv, plotRealZhou)

**## Grid of seven graphs:**

O <- ggplot(data = Tonkdf) +

geom\_point(aes(x = k, y = Tonk\_Act, color = "Actual"), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Tonk\_Exp, color = "Expected"), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") + scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1])) +

geom\_line(aes(x = k, y = Tonk\_Act, color = "Actual")) +

geom\_line(aes(x = k, y = Tonk\_Exp, color = "Expected"))

legend <- get\_legend(O)

graph7 <- plot\_grid(plotlist[[1]], plotlist[[2]], plotlist[[3]], plotlist[[4]], plotlist[[5]], plotlist[[6]], plotlist[[7]], NULL, ncol=3, nrow=3, align="hv", rel\_widths = c(1, 1, 1, 1, 1, 1, 1, 1, 1), labels = c('A', 'B', 'C', 'D', 'E', 'F', 'G', "", ""))

dev.new(width = 9, height = 9)

graph7legend <- graph7 + draw\_grob(legend, 2/3, 0, 1/3, 0.5)

graph7legend

ggsave("FigureI\_real.pdf", plot = graph7legend, device = "pdf", width = 9, height = 7, units = "in", dpi = 300, path = mypath)

**## Second part WITH SYSTEMATIC ERROR ESTIMATED EMPIRICALLY FROM MEANS:**

**## two methods - Range using max - min or Variance of group means**

## Bessel correction gives standard unbiased estimator of the variance of a sample of means and between-group variance corresponding to systematic variability here.

## Sokal RR and Rohlf 2012.

## Montgomery 2017.

global\_mean\_CI\_sys <- function(means, ss, s\_method = c("sd", "SE"), ns, alpha = 0.05, sys\_method = c("var", "range")) {

sys\_method <- match.arg(sys\_method)

s\_method <- match.arg(s\_method)

k <- length(means)

total\_n <- sum(ns)

weights <- ns / total\_n

global\_mean <- sum(weights \* means)

if (s\_method == "sd") {

sds <- ss

var\_sampling <- sum((sds^2 / ns) \* (weights^2))

var\_i <- (weights^2 \* sds^2) / ns

} else if (s\_method == "SE") {

SE <- ss

var\_sampling <- sum((weights^2) \* (SE^2))

var\_i <- (weights^2 \* SE^2)

}

if (sys\_method == "var") {

if (k > 1) {

var\_sys <- sum((means - global\_mean)^2) / (k - 1) ## Bessel correction

} else {

var\_sys <- 0

}

} else if (sys\_method == "range") {

delta <- max(means) - min(means)

var\_sys <- (delta / 2)^2

}

var\_total <- var\_sampling + var\_sys

SE\_total <- sqrt(var\_total)

df <- var\_sampling^2 / sum((var\_i^2) / (ns - 1)) ## Welch's adjustment

t\_crit <- qt(1 - alpha / 2, df)

CI\_lower <- global\_mean - t\_crit \* SE\_total

CI\_upper <- global\_mean + t\_crit \* SE\_total

CI\_sys\_width <- 2\*t\_crit \* SE\_total

CI\_expected <- qt((1-alpha/2), df = sum(ns-1)) / sqrt(k)

return(c(CI\_sys\_width = CI\_sys\_width, CI\_expected = CI\_expected, var\_sampling = var\_sampling, var\_sys = var\_sys))

}

**## A2. Tonk - Systematic error estimate**

k <- 1:7

xbreaks <- c(1, 3, 5, 7)

xlabels <- paste0(xbreaks)

n <- 100

Tonk\_CIdatasysvar <- list();

for (i in 1:nrow(Tonk\_raw\_df)) {

Tonk\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Tonk\_mean[1:i], ss = Tonk\_sd[1:i], s\_method = "sd", ns = Tonk\_n[1:i], sys\_method = "var")

}

Tonk\_CIdfsysvar <- as.data.frame(list.rbind(Tonk\_CIdatasysvar))

Tonk\_CIdfsysvar

colnames(Tonk\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Tonk\_CIdfsysvar

xxx <- Tonk\_CIdfsysvar

xxx

Tonk\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Tonk\_actual\_ratiosysvar

Tonk\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Tonk\_expected\_ratiosys

Tonkdfsys <- data.frame(k, Tonk\_actual\_ratiosysvar, Tonk\_expected\_ratiosys)

colnames(Tonkdfsys) <- c("k", "Tonk\_Actsysvar", "Tonk\_Exp")

Tonkdfsys

Tonkdfsys$Tonk\_Act <- Tonkdf$Tonk\_Act

Tonkdfsys

annotation1 <- data.frame(x = c(4), y = c(200), label = paste0("n = ", n))

annotation1

plotRealTonksys <- ggplot(data = Tonkdfsys) +

geom\_point(aes(x = k, y = Tonk\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Tonk\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Tonk\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Tonk\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Tonk\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Tonk\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") + theme(legend.position = "none")

dev.new()

plotRealTonksys

ggsave("FigureH\_Tonk\_real.pdf", plot = plotRealTonksys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## B2. Kich - Systematic error estimate**

k <- 1:4

n <- 5

xbreaks <- k

xlabels <- paste0(xbreaks)

Kich\_CIdatasysvar <- list();

for (i in 1:nrow(Kich\_raw\_df)) {

Kich\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Kich\_mean[1:i], ss = Kich\_SE[1:i], s\_method = "SE", ns = Kich\_n[1:i], sys\_method = "var")

}

Kich\_CIdfsysvar <- as.data.frame(list.rbind(Kich\_CIdatasysvar))

Kich\_CIdfsysvar

colnames(Kich\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Kich\_CIdfsysvar

xxx <- Kich\_CIdfsysvar

xxx

Kich\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Kich\_actual\_ratiosysvar

Kich\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Kich\_expected\_ratiosys

Kichdfsys <- data.frame(k, Kich\_actual\_ratiosysvar, Kich\_expected\_ratiosys)

colnames(Kichdfsys) <- c("k", "Kich\_Actsysvar", "Kich\_Exp")

Kichdfsys

Kichdfsys$Kich\_Act <- Kichdf$Kich\_Act

Kichdfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

plotRealKichsys <- ggplot(data = Kichdfsys) +

geom\_point(aes(x = k, y = Kich\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Kich\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Kich\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Kich\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Kich\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Kich\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealKichsys

ggsave("FigureH\_Kich\_real.pdf", plot = plotRealKichsys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## C2. Carl - Systematic error estimate**

k <- 1:3

n <- 10

xbreaks <- k

xlabels <- paste0(xbreaks)

Carl\_CIdatasysvar <- list();

for (i in 1:nrow(Carl\_raw\_df)) {

Carl\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Carl\_mean[1:i], ss = Carl\_sd[1:i], s\_method = "sd", ns = Carl\_n[1:i], sys\_method = "var")

}

Carl\_CIdfsysvar <- as.data.frame(list.rbind(Carl\_CIdatasysvar))

Carl\_CIdfsysvar

colnames(Carl\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Carl\_CIdfsysvar

xxx <- Carl\_CIdfsysvar

xxx

Carl\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Carl\_actual\_ratiosysvar

Carl\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Carl\_expected\_ratiosys

Carldfsys <- data.frame(k, Carl\_actual\_ratiosysvar, Carl\_expected\_ratiosys)

colnames(Carldfsys) <- c("k", "Carl\_Actsysvar", "Carl\_Exp")

Carldfsys

Carldfsys$Carl\_Act <- Carldf$Carl\_Act

Carldfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

plotRealCarlsys <- ggplot(data = Carldfsys) +

geom\_point(aes(x = k, y = Carl\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Carl\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Carl\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Carl\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Carl\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Carl\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealCarlsys

ggsave("FigureH\_Carl\_real.pdf", plot = plotRealCarlsys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## D2. Lawr - Systematic error estimate**

k <- 1:3

n <- 5

xbreaks <- k

xlabels <- paste0(xbreaks)

Lawr\_CIdatasysvar <- list();

for (i in 1:nrow(Lawr\_raw\_df)) {

Lawr\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Lawr\_mean[1:i], ss = Lawr\_SE[1:i], s\_method = "SE", ns = Lawr\_n[1:i], sys\_method = "var")

}

Lawr\_CIdfsysvar <- as.data.frame(list.rbind(Lawr\_CIdatasysvar))

Lawr\_CIdfsysvar

colnames(Lawr\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Lawr\_CIdfsysvar

xxx <- Lawr\_CIdfsysvar

xxx

Lawr\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Lawr\_actual\_ratiosysvar

Lawr\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Lawr\_expected\_ratiosys

Lawrdfsys <- data.frame(k, Lawr\_actual\_ratiosysvar, Lawr\_expected\_ratiosys)

colnames(Lawrdfsys) <- c("k", "Lawr\_Actsysvar", "Lawr\_Exp")

Lawrdfsys

Lawrdfsys$Lawr\_Act <- Lawrdf$Lawr\_Act

Lawrdfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

plotRealLawrsys <- ggplot(data = Lawrdfsys) +

geom\_point(aes(x = k, y = Lawr\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Lawr\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Lawr\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 265)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Lawr\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Lawr\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Lawr\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealLawrsys

ggsave("FigureH\_Lawr\_real.pdf", plot = plotRealLawrsys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## E2. Zhan - Systematic error estimate**

k <- 1:3

n <- 10

xbreaks <- k

xlabels <- paste0(xbreaks)

Zhan\_CIdatasysvar <- list();

for (i in 1:nrow(Zhan\_raw\_df)) {

Zhan\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Zhan\_mean[1:i], ss = Zhan\_SE[1:i], s\_method = "SE", ns = Zhan\_n[1:i], sys\_method = "var")

}

Zhan\_CIdfsysvar <- as.data.frame(list.rbind(Zhan\_CIdatasysvar))

Zhan\_CIdfsysvar

colnames(Zhan\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Zhan\_CIdfsysvar

xxx <- Zhan\_CIdfsysvar

xxx

Zhan\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Zhan\_actual\_ratiosysvar

Zhan\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Zhan\_expected\_ratiosys

Zhandfsys <- data.frame(k, Zhan\_actual\_ratiosysvar, Zhan\_expected\_ratiosys)

colnames(Zhandfsys) <- c("k", "Zhan\_Actsysvar", "Zhan\_Exp")

Zhandfsys

Zhandfsys$Zhan\_Act <- Zhandf$Zhan\_Act

Zhandfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

plotRealZhansys <- ggplot(data = Zhandfsys) +

geom\_point(aes(x = k, y = Zhan\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Zhan\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Zhan\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 260)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Zhan\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Zhan\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Zhan\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealZhansys

ggsave("FigureH\_Zhan\_real.pdf", plot = plotRealZhansys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## F2. Driv - Systematic error estimate**

k <- 1:3

n <- 3

xbreaks <- k

xlabels <- paste0(xbreaks)

Driv\_CIdatasysvar <- list();

for (i in 1:nrow(Driv\_raw\_df)) {

Driv\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Driv\_mean[1:i], ss = Driv\_SE[1:i], s\_method = "SE", ns = Driv\_n[1:i], sys\_method = "var")

}

Driv\_CIdfsysvar <- as.data.frame(list.rbind(Driv\_CIdatasysvar))

Driv\_CIdfsysvar

colnames(Driv\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Driv\_CIdfsysvar

xxx <- Driv\_CIdfsysvar

xxx

Driv\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Driv\_actual\_ratiosysvar

Driv\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Driv\_expected\_ratiosys

Drivdfsys <- data.frame(k, Driv\_actual\_ratiosysvar, Driv\_expected\_ratiosys)

colnames(Drivdfsys) <- c("k", "Driv\_Actsysvar", "Driv\_Exp")

Drivdfsys

Drivdfsys$Driv\_Act <- Drivdf$Driv\_Act

Drivdfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

plotRealDrivsys <- ggplot(data = Drivdfsys) +

geom\_point(aes(x = k, y = Driv\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Driv\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Driv\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 260)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Driv\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Driv\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Driv\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealDrivsys

ggsave("FigureH\_Driv\_real.pdf", plot = plotRealDrivsys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## G2. Zhou - Systematic error estimate**

k <- 1:4

n <- 3

xbreaks <- k

xlabels <- paste0(xbreaks)

Zhou\_CIdatasysvar <- list();

for (i in 1:nrow(Zhou\_raw\_df)) {

Zhou\_CIdatasysvar[[i]] <- global\_mean\_CI\_sys(means = Zhou\_mean[1:i], ss = Zhou\_sd[1:i], s\_method = "sd", ns = Zhou\_n[1:i], sys\_method = "var")

}

Zhou\_CIdfsysvar <- as.data.frame(list.rbind(Zhou\_CIdatasysvar))

Zhou\_CIdfsysvar

colnames(Zhou\_CIdfsysvar) <- c("CI\_sys\_widthvar", "CI\_expected", "var\_sampling", "var\_sys\_var")

Zhou\_CIdfsysvar

xxx <- Zhou\_CIdfsysvar

xxx

Zhou\_actual\_ratiosysvar <- 100 \* signif(xxx$CI\_sys\_widthvar / xxx[2, "CI\_sys\_widthvar"], 4)

Zhou\_actual\_ratiosysvar

Zhou\_expected\_ratiosys <- 100 \* signif(xxx$CI\_expected / xxx[2, "CI\_expected"], 4)

Zhou\_expected\_ratiosys

Zhoudfsys <- data.frame(k, Zhou\_actual\_ratiosysvar, Zhou\_expected\_ratiosys)

colnames(Zhoudfsys) <- c("k", "Zhou\_Actsysvar", "Zhou\_Exp")

Zhoudfsys

Zhoudfsys$Zhou\_Act <- Zhoudf$Zhou\_Act

Zhoudfsys

annotation1 <- data.frame(x = c(2), y = c(200), label = paste0("n = ", n))

annotation1

ybreaks <- c(0, 100, 200, 300, 400)

ylabels <- paste0(ybreaks)

plotRealZhousys <- ggplot(data = Zhoudfsys) +

geom\_point(aes(x = k, y = Zhou\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Zhou\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Zhou\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 400)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Zhou\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Zhou\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Zhou\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold") +

theme(legend.position = "none")

dev.new()

plotRealZhousys

ggsave("FigureH\_Zhou\_real.pdf", plot = plotRealZhousys, device = "pdf", width = 6, height = 7, units = "in", dpi = 300, path = mypath)

**## All Real data: Systematic:**

mylistsys <- list(Tonkdfsys, Kichdfsys, Carldfsys, Lawrdfsys, Zhandfsys, Drivdfsys, Zhoudfsys)

mylistsys

plotlistsys <- list(plotRealTonksys, plotRealKichsys, plotRealCarlsys, plotRealLawrsys, plotRealZhansys, plotRealDrivsys, plotRealZhousys)

**## Grid of seven graphs:**

Osys <- ggplot(data = Tonkdfsys) +

geom\_point(aes(x = k, y = Tonk\_Actsysvar), size = 3, shape = 1) +

geom\_point(aes(x = k, y = Tonk\_Act), size = 3, shape = 3) +

geom\_point(aes(x = k, y = Tonk\_Exp), size = 3, shape = 2) +

scale\_y\_continuous(breaks = ybreaks, labels = ylabels, expand = c(0, 0), limits = c(0, 230)) + ylab("C.I.(%)") +

scale\_x\_continuous(breaks = xbreaks, labels = xlabels, expand = c(0, 0), limits = c(1, max(k) + 0.2)) +

scale\_colour\_manual(name="Ratio", values = c(cbp2[8], cbp2[1], cbp2[6], cbp2[5])) +

scale\_linetype\_manual(name="Ratio", values=c("Actualsys" = "dotted", "Actual" = "dashed", "Expected" = "solid")) + theme(axis.title.x = element\_text(vjust = -2)) +

geom\_line(aes(x = k, y = Tonk\_Actsysvar, color = "Actualsys", linetype = "Actualsys")) +

geom\_line(aes(x = k, y = Tonk\_Act, color = "Actual", linetype = "Actual")) +

geom\_line(aes(x = k, y = Tonk\_Exp, color = "Expected", linetype = "Expected")) +

geom\_text(data = annotation1, aes(x=x, y=y, label=label), color = cbp2[8], size=5, fontface="bold")

legendsys <- get\_legend(Osys)

graph7sys <- plot\_grid(plotlistsys[[1]], plotlistsys[[2]], plotlistsys[[3]], plotlistsys[[4]], plotlistsys[[5]], plotlistsys[[6]], plotlistsys[[7]], NULL, ncol=3, nrow=3, align="hv", rel\_widths = c(1, 1, 1, 1, 1, 1, 1, 1, 1), labels = c('A', 'B', 'C', 'D', 'E', 'F', 'G', "", ""))

dev.new(width = 9, height = 9)

graph7legendsys <- graph7sys + draw\_grob(legendsys, 2/3, 0, 1/3, 0.5)

graph7legendsys

ggsave("FigureJ\_realSysErrpdf", plot = graph7legendsys, device = "pdf", width = 9, height = 9, units = "in", dpi = 300, path = mypath)

**## CODING REFERENCES**

packages <- c("effectsize", "rcompanion", "pwr", "purrr", "broom", "DescTools", "Hmisc", "XNomial", "rlist", "plyr", "cowplot", "ggtext", "ggarrow", "ggforce", "forcats", "dplyr", "data.table", "ggplot2", "ggpattern", "tidyverse")

**## References:**

**R packages:** effectsize(1), rcompanion(2), pwr(3), purrr(4), broom(5), DescTools(6), Hmisc(7), XNomial(8), rlist(9), plyr(10), cowplot(11), ggtext(12), ggarrow(13), ggforce(14), forcats(15), dplyr(16), data.table(17), ggplot2(18), ggpattern(19), tidyverse(20),

**Data sources:** Tonk et al. 2017(21), Kichigina et al. 2017(22), Carlucci et al. 2017(23), Lawrence et al. 2017(24), Zhang et al. 2017(25), Driver et al. 2017(26), Zhou et al. 2017(27),

**General statistics:** Johnson et al. 1995(28), Ruxton 2006(29), Ballico 2000(30), Welch 1947(31), Sokal and Rohlf 2012(32), Montgomery 2017(33), Cohen(34)

**Power studies:** Clark et al.(35)

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